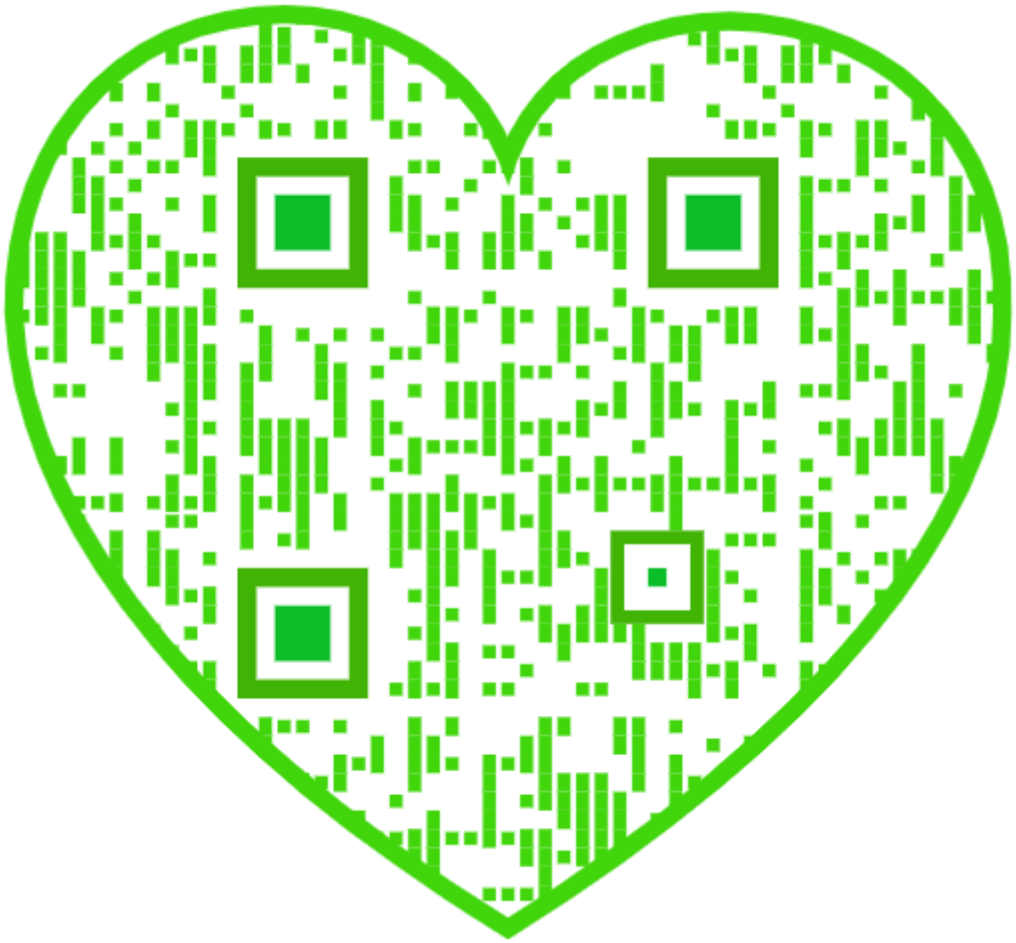

HANDBOOK

OF

PRACTICAL BOTANY

FOR THE BOTANICAL LABORATORY AND PRIVATE STUDENT.

BY

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AND WITH MANY ADDITIONAL NOTES BY AUTHOR AND EDITOR

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PREFACE BY THE AUTHOR.

THIS book is intended chiefly for those who, without desiring to become botanists by profession, wish nevertheless to become acquainted with the elements of scientific structural botany. It will likewise introduce the beginner to the various methods of microscopical manipulation.

The study of vegetable structure is especially favourable as an initiation into the use of the microscope; and any one whose future career will require command over this instrument should commence with the study under the microscope of vegetable anatomy.

The manual is divided into thirty-two chapters, each of which is intended to provide materials for several hours' practical work in the laboratory. The earlier chapters are easy, and the difficulties to be encountered increase almost continuously up to the last chapter. The first chapter assumes on the part of the worker entire ignorance as to the use of his instruments, but nevertheless assumes the possession of some general botanical knowledge. With this elementary preparation the beginner ought to be able, by the diligent use of this book alone, to acquire a tolerably broad knowledge both of vegetable structure and of the methods of microscopical work.

The objects for study have been so selected that most can be obtained with comparative ease. In many places I have recommended the use of plants preserved in alcohol, as the worker is thus rendered independent of the time of year. As, however, the objects may need to be collected even months

before being used, the student ought carefully to consult a special list of plants or portions of plants needed for his work, and which ought to be collected at some given time or condition. Not infrequently the objects need to undergo, in order to make them fit for use, some preliminary preparation, which may take several hours, or even a day. The student ought therefore to take cognisance of a lesson a sufficiently long time before commencing work.

The list of necessary reagents will be found at the end of the book. These reagents should be ready before beginning work. The method of preparation of special reagents for histological work is also given in this list. Many of these it is preferable to obtain ready-made from the firm mentioned at the head of the list.

The explanations and illustrations of the use of the instruments and reagents are scattered in the text; but the general index is made so far complete as to enable the student easily to refer to any explanations which may be necessary.

I have given especial care to the methods of study of the Bacteria; with the preparation which this book gives, the student will be capable of following out any deeper researches into this subject, as well as of realizing their practical application.

All the figures in this work have been drawn by myself from nature. Almost all of the facts given in the text, even those which were well known, have been submitted to careful control.

At the close of each chapter are given some bibliographical notes, which show the student the fountain-head whence fuller information can be obtained.

ED. STRASBURGER.

PREFACE TO THE ENGLISH EDITION.

ALTHOUGH the last two or three years have produced at least as many works on Practical Botany for Laboratory purposes, no apology is needed for reproducing from the German one which has no counterpart in the English language, and which has the advantage of being written by one of the greatest living masters of microscopical observation.

Although Professor Strasburger has revolutionized the science of Botany in more than one direction, no work of his has as yet come before an English public in its own tongue; but it is perhaps not unfitting that the Author's first introduction to the English-reading student should be in the rôle of teacher of those arts of manipulation and observation by the exercise of which his own fame has been gained.

This edition has the advantage of revision and of numerous additional notes by the Author; some portions have been well-nigh rewritten. To these I have ventured to add notes of my own, intended to either simplify or amplify the description, or to enable the material selected by the Author to be replaced by some other, probably more readily obtainable. These additions have been either inserted as footnotes, or, where intercalated in the text, are usually inserted between square brackets [ ]. The Introduction I have, with the consent of the Author, nearly rewritten, in order to make it more suited to the English student; similarly with a few other isolated paragraphs. A few additional illustrations I have been enabled to add, through the courtesy of the Publishers; descriptions of, and references to, these are likewise enclosed in brackets. To make the book more convenient in use, I

have given at the head of each chapter (task, or lesson, in the original) a list of the objects required for study in that chapter. I regret that I did not add to these lists any special reagents which might be required for use; possibly a future edition may give opportunity for this.

I have considerably enlarged the scope of Appendices II. and III., and have added two new Appendices, I. and IV., which I hope may be useful to the student. Throughout the work I have likewise added the common English names (if any) of the plants referred to.

The student will probably not be able to carry out all the investigations constituting a chapter at the same time. A careful note should be made of any which are thus postponed, so that they may be taken up in due season. It is not unlikely that some may not come at all within the range of the student's observation; for these examples the book must be looked upon in the light of a text-book.

The student is earnestly urged to study from the beginning the Author's methods of work. These are especially noteworthy when he comes, perhaps incidentally, to correlate structure with function. The interdependence of these two factors in the plant's life history is the great underlying principle of modern botanical teaching, and the student cannot too soon begin to exercise his thoughts in this direction, resting assured that his methods are right even though his results may for the time being prove to be erroneous.

As to translation, no one can feel so fully as myself its many and serious defects. I can only plead that the work was executed at a time of serious pressure, and, although circumstances have delayed the issue of the book, the manuscript was out of my hands, and therefore only subject to such limited correction as proof-sheets would allow.

W. H.

BIRMINGHAM, *September*, 1886.

PREFACE

TO THE

SECOND ENGLISH EDITION.

ALTHOUGH anything like a complete revision of the text has been impracticable, I have been enabled, through the liberality of the Publishers, to make several considerable and important additions both to the text and figures (mainly derived from the Second Edition of the *Botanisches Practicum*), and the chapters upon "Vascular Bundles" and "Bacteria" have been to some extent re-written. A list of the most important of these changes will be found on page xii., at the end of the Contents. I hope that these additions will still further enhance the utility of the work to those for whom it is intended. I have likewise made a few verbal and other corrections, and, after much hesitation, have eliminated the square brackets wherever this has been feasible.

I have most heartily to thank many correspondents here, in America, and elsewhere, for numerous notes, memoranda, and criticisms. Although I may not at present have been able to make use of them, these suggestions will not be lost sight of; and even should they never be used, I am none the less grateful for the kindly spirit which has prompted their transmission.

W. H.

August 1, 1889.

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The most important changes and additions in the Second Edition are the following:—

CHAPTER

VII. New Figure, Glandular hairs of *Viola tricolor* (36A) on page 82*a*, and descriptive text.

VIII. New Figure, 41, page 90, replaces similar figure in Ed. i.

IX. New Figure, 45, p. 101, replaces similar figure in Ed. i., and similarly with new Figure, 46, p. 107. New Figure, 45**, p. 105, cross-section through twig of *Aristolochia Sipho*. New Figure, 46, p. 107, replaces similar figure in Ed. i. This chapter has been in great part re-written.

XI. New Figure, 50A, p. 126, cross-section through the wood of *Tilia parvifolia*; new Figure, 50B, p. 127, cross-section through the bast of the same, with accompanying text.

XXI. New Figure, 84*, p. 232, Bacteria of the fur of teeth, and many alterations and additions to the text.

XXII. New Figure, 87*, p. 254*b*, Reproduction of *Fucus platycarpus* and *F. vesiculosus*; and new Figure, 87**, p. 254*h*, Reproduction of *Chara fragilis*; and accompanying text.

XXIII. New Figure, 87C, p. 256*b*, Spore formation in *Mucor Mucedo*; and new Figure, 87D, p. 256*d*, Zygote formation and germination in the same; and accompanying text.

XXVII. New Figure, 102A, p. 310*a*, Embryo-sac and fertilization of *Picea vulgaris*; new Figure, 102B, p. 310*c*, embryology of the same; new Figure, 102c, p. 310*c*, later embryology of the same; and accompanying text.

XXVIII. New Figure, 104A, p. 320, Pollen-grains of *Malva crispa*; new Figure, 104B, p. 320*b*, Pollen-grains of *Cucurbita*; and accompanying text.

XXX. New Figure, 112A, p. 346*b*, Structure of grain and embryo of wheat, and accompanying text.

INTRODUCTION.

STUDENTS at Universities, or properly equipped Colleges, or Schools of Science, will usually find in the Botanical Laboratory the instruments which are needed for their work. For those, however, who are not connected with such an institution, but may use this book independently as an introduction to the practical study of the minute structure of plants, as well as those who, under any circumstances, wish to become possessed of suitable instruments for microscopical work, the following lists, selected from the most recent catalogues of opticians, may be of service. The first list here following includes microscopes, with a price (affixed) ranging up to about £8.

I.—*English and American Makers.* Some of these, such as the well-known firms of Ross & Co., Powell & Lealand, R. & J. Beck, and Zentmayer, are notably dearer than many other English and American, and than most foreign makers, and therefore probably for student purposes are less available. The microscopes built on the so-called "English model" are more massive and complicated in their construction than is really necessary for student purposes, and the object on the stage is usually moved about by means of a mechanical arrangement of screws, where, for ordinary purposes, the fingers had far better be used. Further, though the diameter of the body of the English microscope may be an advantage, its length is doubtfully so, and renders the erect position of the instrument in working, which is for most purposes far the best (though a joint permitting inclination is highly desirable), almost impossible. The distance of the stage from the eye renders delicate working with the fingers a matter of some difficulty; for it is notorious that the nearer the fingers are to the eyes, within certain limits, the more delicately their movements can be controlled. These makers have, however, recognised the need of instruments of more compact form, simpler construction, and

lower price, and, like the cheaper English makers hereafter noted, have brought out instruments suited for general use under the name either of "Student's," "Educational," or "Economic" microscopes. Of such kind we will specially indicate a few.* These will be exclusively monocular; binocular microscopes are in no way needed.

Ross & Co. (112, New Bond Street, London) produce an instrument of high quality and comparatively low price, called the "Student's Microscope," with rotating glass stage, coarse and fine adjustment by screws, and a "swinging tail-piece for oblique illumination," originally devised by Zentmayer, of Philadelphia, into which may be fixed various substage appliances, such as condenser, etc. Price, with one eye-piece (A), and in mahogany case, £10 10*s*. Another cheaper stand is the "Student's Monocular Stand, No. 1," with coarse adjustment by sliding the tube, and fine adjustment by a screw, circular rotating glass stage, and draw-tube into which the continental eye-pieces will fit. (This is the case also with the above higher-priced instrument.) With one eye-piece the price of this stand is £4 10*s*.; a diaphragm to the stage costs 8*s*., and a case for the microscope 11*s*. extra. Ross's best objectives are too expensive for ordinary student use; he offers some for the above microscope—*e.g.* 1 inch of 15° angular aperture at £1 5*s*., and ¼ of 75° for £2 2*s*.; or, together with the stand, as above, £8 16*s*., to which ought to be added a second eye-piece, which costs £1. For 15*s*. extra this microscope can be obtained with screw coarse adjustment. Instead of these objectives, the stand as above can be fitted with objectives by other makers.†

R. & J. Beck (68, Cornhill, London, E.C.) offer a useful instrument under the name of the "Monocular Economic Microscope" (No. 24, Catalogue 1885), having coarse adjustment by sliding tube, fine do. by screw, drawtube, 1 eyepiece, diaphragm,

* For further information and particulars the reader is referred to any of the current works on the microscope, such as those of Dr. Lionel Beale, Dr. Jabez Hogg, and, especially, that of the late Dr. Carpenter.

† It is here perhaps desirable to note that all English objectives are made with the same size screw, the "Microscopical Society's Screw," known abroad as the "English Screw," and therefore will fit any English instrument. Foreign objectives, if instructions are given to that effect, are likewise made with this screw, usually with an extra charge of but a shilling or two, sometimes none. Foreign microscopes can be made with the same gauge, or provided with adaptors. It is not improbable, and greatly desirable, that the English, or some other standard, screw may become universal, as the confusion amongst foreign makers is extreme. At the same time all of, at least, the smaller English microscopes would be better made for eye-pieces of continental size.

1 inch and $\frac{1}{4}$ inch object glasses, in mahogany case, for £5 5*s*. Without objectives, but with 2 eye-pieces, £3 10*s*. To this can be fitted an achromatic condenser for £1 2*s*., and other pieces of apparatus. Beck's best objectives are expensive; but he constructs good student glasses at a lower rate.

Amongst the cheaper optical firms, we may mention the following:—

C. Baker (244 & 245, High Holborn, London, W.C.) publishes a "Medical Microscope" on the old continental model (of Nachet & Hartnack), with draw-tube, coarse adjustment by sliding tube, fine by screw, and 2 eye-pieces, in mahogany case, for £3 3*s*. With 1 inch and $\frac{1}{4}$ inch object-glasses and condenser for opaque objects, £6 7*s*.

Chas. Collins (157, Gt. Portland Street, Oxford Street, London, W.) offers a "Histological Microscope," with coarse adjustment by rackwork *or* by sliding tube, fine screw adjustment, one eye-piece, 1 inch and $\frac{1}{4}$ *or* $\frac{1}{5}$ objectives, in mahogany case, for £5 10*s*.; or with extra eye-piece, polariscope and stage condenser, for £7 10*s*. Also a "Student's Microscope" of rather larger size, with similar fittings, at an extra cost of £1 10*s*.

H. Crouch (66, Barbican, London, E.C.) publishes "The Histologist's Microscope," coarse adjustment by sliding in cloth-lined tube, fine by screw, glass stage with diaphragm, 1 inch and $\frac{1}{3}$ or $\frac{1}{4}$ inch objectives and 2 eye-pieces, in mahogany case, for £5 5*s*. A Stand Condenser can be added for 8*s*. 6*d*., and an Achromatic Condenser for £1 1*s*.

T. Darton & Co. (45, St. John's Street, West Smithfield, London, E.C.) have an "Improved Histological Microscope," on much the same model as that of Crouch, with draw-tube, screw fine adjustment, 2 eye-pieces, $\frac{1}{2}$ inch and $\frac{1}{6}$ inch objectives, glass stage, in mahogany cabinet, for £5 5*s*. Other apparatus can be fitted.

Parkes & Son (St. Mary's Row, Birmingham) offer a "Portable Educational Microscope," a reliable and very steady instrument, with coarse adjustment by body sliding in cloth-lined tube, fine by screw, draw-tube, 2 eye-pieces, 1 inch objective, separating to 2 inches, $\frac{1}{4}$ inch ditto, with magnifying power ranging, with use of draw-tube, from 140 to 470 diameters; also with spot lens, condenser on jointed arm attached to the stand, diaphragm, with disc for "white-cloud illumination," and glass stage, in mahogany case with leather handle, £6 10*s*. If with $\frac{1}{6}$ inch objective instead

of $\frac{1}{4}$, increasing magnifying power to 560 diameters, 5*s.* extra; or, instead, with $\frac{1}{7}$ inch, magnifying up to 700 diameters, £7. The object-glasses are provided with a "patent sliding adapter," obviating the necessity for screwing in exchanging one glass for another while at work. A screw nozzle can also be had to adapt it for all other objectives with the English screw. An achromatic condenser can be supplied adapted for it.

PILLISCHER (New Bond Street, London), under the name of "International Microscope," offers a stand on the old continental model, but with rackwork coarse adjustment, 2 eye-pieces, $\frac{5}{8}$ and $\frac{1}{7}$ inch objectives, giving, with draw-tube, a range of from 50 to 420 diameters, in case, for £7 10*s.*

SWIFT & SON (81, Tottenham Court Road), under the name of the "College Microscope, No. 1," offer an instrument with coarse adjustment by sliding in cloth-lined tube, fine by screw, draw-tube (too large for continental eye-pieces), diaphragm, 1 eye-piece, 1 inch and $\frac{1}{4}$ inch objectives, in case, for £5 5*s.* The same with screw coarse adjustment, glass stage, and jointed mirror, £1 10*s.* extra. Achromatic condenser, 12*s.* to £1 5*s.*

Other instruments of equal excellence with the above are doubtless manufactured; there is no pretence that this list is complete, nor is any comparison intended to be instituted.

German Makers. Of these, the following two makers may be considered typical:—

CARL ZEISS (Jena). Stand VIIa, with 3 eye-pieces, Nos. 2, 4, and 5, and objectives B and D, price £7 13*s.* This stand has an unjointed back, sliding tube for coarse and screw for fine adjustment, and swinging mirror. The instrument gives a magnification from 95 to 580 diameters. The glasses of Zeiss are unsurpassed.

E. LEITZ (Wetzlar). A stand of similar model to that of Zeiss, with eye-pieces I, and III, and objectives 3 and 7 (No. 17 in catalogue of 1882), and magnifying from 80 to 500 diameters, price £5 10*s.* The objectives of Leitz are low in price, but remarkably good.

Other makers are SEIBERT (Wetzlar), BÉNÈCHE (Berlin), HARTNACK (Potsdam), WINKEL (Göttingen), PLÖSL & Co. (Vienna), REICHERT (Vienna); all good.

French Makers. The two following are probably the best:—

BEZU, HAUSSER & Co. (Paris, Rue Bonaparte, 1, successors to the old house of Prazmowski, formerly Hartnack & Prazmowski), Stand VIII., eye-pieces 2 and 4, objectives 4 and 8, magnifying

50 to 600 diameters, price about £8. Stand VIIIA, the same as above, but with jointed back, 12*s.* extra.

C. VÉRICK* (Paris, Rue de la Parcheminerie, 2). Stand VI., with jointed back, with diaphragm disk and draw-tube, 2 eye-pieces, 1 and 3, 2 objectives, 2 and 6, magnifying 60 to 500 diameters, price 165 francs (about £6 12*s.*); or Stand IV., with which achromatic condenser and polariscope can be used, about £2 more. These two instruments are now very widely used in France.

Most, or all, of the above makers, English and foreign, manufacture microscope stands of cheaper quality than the above; it must, however, be borne in mind that accurate observation needs an instrument which is capable of it, and while there is, and ought to be, every desire to keep the cost within a reasonably small sum, true economy does not consist in purchasing an instrument which may be a constant source of dissatisfaction, and may have to be discarded when the student emerges from his swaddling clothes. The stand which is purchased ought to be adapted to the receipt of optical apparatus other than the simple eye-piece and objective. It should have a jointed back, and be thoroughly steady in any position; the adjustment should be easy and true, and if the body is twisted, any object observed should not be thrown out of centre; the mirror should be plane and concave, and should have a long jointed arm; and the stage should be constructed for the reception of a condenser. Still more essential is it that the special optical parts, the eye-pieces and objectives, should be good. They should let through the largest possible amount of light (the diaphragm will easily control its quantity if needed), and there should be a complete absence of colour, both round the exterior of the field of view, and round any object, or particles of dust, in focus. The field should be *flat*, so that a small object moved from one part to another alters neither in distinctness, form, nor size. Lastly, the objective should have a fair working distance from the object, or the thickness of the cover-glass, to be hereafter noted, may become a matter of great importance.

In all these points, except perhaps accurate centering, the stands of English makers either equal or excel, price for price, the foreign stands; while, on the contrary, price for price, the eye-pieces and objectives of continental makers usually are far superior to those made by the English opticians, a superiority probably due solely to the more trained skill and more patient accuracy of the workmen.

* Now Stiasnie.

All of the work in this book, perhaps, with the exception of Chapter XXI., can be performed with the aid of objectives up to ⅓; but the student who has gained some experience will probably wish to add to his microscope one or more stronger objectives, in order to increase the range of his work. Increased magnification can be obtained by increased power either of eye-piece or of object-glass. All the objectives we have heretofore noted are what are called "dry" systems, since they are used for work in a dry state, and a layer of air separates the objective from the object. "Dry" objectives of high power are subject to great disadvantage from the serious loss of light their use involves. The light, in passing from the mirror to the objective, passes in the first place through air; then the object-slide, next the object and the medium in which the object is mounted, which may be glycerine, water, alcohol, etc., or even air; then through the cover-glass, and finally through air again. In every one of these changes light is lost. Owing to this loss of light, as well as for other reasons, it is not wise to use high power eye-pieces with dry objectives, added to which, as the eye-piece does not magnify the object, but only the image of it as given by the objective, any errors of this latter are likewise magnified by the eye-piece.

To obviate in part this loss of light, what are called "immersion" objectives have been for the last few years much in use. In these objectives the cover-glass and the front lens of the object-glass are connected by a drop of liquid. Such objectives are of two kinds: "water," in which that is the liquid used; and "homogeneous," in which the liquid is in general oil, or a mixture of oils, but sometimes is glycerine, etc. The homogeneous immersion objectives are dearer, less readily cleaned after use, and require a supply of the special fluid for which they are manufactured; but on the other hand they transmit more light, bear a higher eye-piece, and are independent in their working of the thickness of the cover-glass. Dry objectives, and water-immersions, of high power are naturally dependent on the thickness of the cover-glass which the light-rays pass through after leaving the object. To provide for this, they are usually manufactured also with "correcting screw," for use according to the thickness of the cover-glass, and at a somewhat increased price. The correcting screw accommodates the objective to the thickness of the cover-glass which happens to be in use, but a right use of it requires considerable experience, nor is it usually needed with any of the weaker immersion systems.

An immersion objective, without correcting screw, is made to suit a certain medium thickness of cover-glass, which is usually stated by the optician, and it is therefore preferable for the beginner, if he wants such an objective, to use with it cover-glasses of this definite thickness.* On the correcting screw, where the system has it, are usually divisions and figures, which allow the focussing for any given thickness of cover-glass, where this is known.† But whoever does not fear the expense would do well to provide himself at once with a system for "homogeneous immersion." They are all constructed without correcting screw, since, as already indicated, the thickness of the cover-glass, of course within the permissible limits, is of no importance. By selecting a single such objective, say $\frac{1}{12}$, and purchasing a series of eye-pieces, one can obtain a range of possible magnification such as could only be given by several water-immersions, or dry objectives. A system for homogeneous immersion, provided it is perfectly constructed, can therefore replace several systems of another kind.‡

Even in the smallest stands mentioned above, objectives for homogeneous immersion can be used with great advantage without any special apparatus for increasing the illumination; but the highest capabilities of the homogeneous system are only brought out by the use of a sub-stage achromatic condenser. Several of the stands referred to above have sub-stage condensers constructed specially for them, and at a cost which, for these small microscopes, would rarely exceed £1 10*s*.

Owing to the prevalent use of the standard screw of the Royal Microscopical Society of London, objectives of one maker can be attached to the instrument of another. Where this screw is not in use by the maker, the objective can have an adaptor attached. A point of some importance to English purchasers of continental objectives is this:—the customary length of the tube of the microscope on the Continent is 150 to 170 millimetres (6 to 7 inches), and the objectives are constructed to suit this length. If the tube exceeds this length, it should be stated in ordering the objectives, that they may be modified to suit. This is especially needed in ordering objectives for homogeneous immersion. All the microscope stands mentioned above have tubes of continental length, and most of continental size.

* On this subject see a note on page xxii.
† Further information on this point in Chap. XXI., p. 224.
‡ Leitz, of Wetzlar, produces a $\frac{1}{12}$ of remarkable excellence, for £6 10*s*.

To give a theory of the formation of the microscopic image does not come within the range of our purpose, and for this we would refer to text-books on Physics and to special works on the microscope. Our task, on the other hand, will consist in making the beginner acquainted with the most important facts of microscopical botany, with the use of the microscope, and with microscopical manipulation. This instruction will be given by studies upon the objects themselves.

Besides the compound microscope to which we have hitherto exclusively referred, a simple, or so-called dissecting, microscope is also more or less necessary. For all the purposes of this book, and, indeed, for most botanical purposes, whether in anatomy or morphology, a dissecting microscope of very simple construction is all that is needed. Most such instruments are unnecessarily complex and expensive. Some, for instance, are constructed to magnify up to 60, 80, or even 100 diameters; if such magnifying power is needed, the low power of the compound microscope will do equally well, dissecting being done upon the stage, but the arms being carefully supported. The following are a few typical simple microscopes, any one of which would suffice:—

Ross & Co.'s "Magnifier Stand," with two lenses of $\frac{1}{2}$-inch and $\frac{1}{4}$-inch focus, magnifying 20 and 40 linear, in flat morocco case, £2 2*s.**

C. Collins, "Dissecting Microscope," with two lenses, to be used together or separately, 15*s.* (No arm-rests.)

Parkes & Son, "Simple Microscope" (No. 5030), on jointed arm, with universal movement, 15*s.**

Swift & Son, "Simple Dissecting Microscope," with three lenses, 18*s.**

Zeiss, "Small Dissecting Microscope" (No. 117, Catalogue 1885), 18*s.*, to which double lenses, magnifying 10, 15, or 30 diameters, at 6*s.* each (Arm-rests).

The student can entirely dispense with a dissecting microscope, and dissect upon the stage of his larger instrument; but as the image of the object is inverted, and any movements he may make are likewise reversed, he would probably be at first somewhat perplexed. Practice will overcome this difficulty; or it can be cleared away at once by purchasing an "erector" for insertion in

* In these instruments the object is dissected on the table, or in any other convenient place. Those not marked have a special stage, with or without arm-rests, as indicated. See also p. 21 *et seq.*

the draw-tube, costing usually 10*s.* or 10*s.* 6*d.* It is desirable likewise to have a low power objective, *e.g.*, 2-inch or 1½-inch, though dissection with the 1-inch is perfectly simple. The lowest power eye-piece should be used. Dissection under the compound microscope has, with very small objects, this further advantage, that there is no chance of losing them in removing from one instrument to the other. To this may be added perhaps another advantage, in that the working-table is not cumbered with an extra instrument. For dissecting with the microscope the wrists must be supported on a level with the object, or slightly below it. Some dissecting microscopes have arm-rests for this purpose; blocks of wood of proper height, or even stacks of books will answer admirably.

A very necessary adjunct for microscopical work is a good magnifying lens, as it is often desirable first of all to study an object with it, afterwards using the microscope. The lenses of the dissecting microscope can be used as hand magnifiers, and low power objectives likewise make good hand lenses. It is worth while, however, to get a lens magnifying about six diameters; very convenient are the triplets, three lenses in a tortoise-shell case, usable separately or together, and sold at a price of about 3*s.* 6*d.* Remarkably beautiful are the Platyscopic Lenses of Browning (63, Strand, London, W.C.), magnifying 15, 20, or 30 diameters, price 18*s.* 6*d.* each, and the Aplanatic Lenses of Zeiss, magnifying 6, 10, or 20 diameters, price 12*s.* or 15*s.* each.

As it is desirable that the student should from the first begin to draw the objects he examines (practical instruction in which will be found on p. 30 *et seq.*), it is desirable that he should have some form of drawing instrument to facilitate his work. Drawing can, it is true, be done without any such aid, but is more difficult. An apparatus for drawing (camera lucida) is constructed either for use with the body of the microscope placed horizontally, or placed vertically. Practically the latter is much preferable. Every microscope maker has appliances of his own make, but they vary very much in real utility. Probably the best in existence are two made by Zeiss, the new camera lucida of Abbe, price 30*s.*, or the camera lucida with two prisms, price 21*s.* The former is specially constructed for the eye-piece No. 2 of Zeiss, and is mounted upon it; it permits drawing upon a horizontal surface; during observation it is removed. The second is slipped by means of a ring upon the tube or the eye-piece (any eye-piece of continental size); it

requires an inclined surface for drawing, but has, however, the advantage that it is always kept upon the microscope, and during the observation is only pushed on one side. Both apparatus require drawing desks, Abbe's camera a horizontal one, the drawing prism one inclined about 25°. The height of the desk should in general correspond with the height of the stage of the microscope. In specially long or shortsighted observers, it should be arranged according to the distance of distinct vision.

Most English opticians supply drawing prisms of one kind or another, capable of satisfactory work. None, however, in my experience, equal those of Zeiss.

A stage micrometer is likewise necessary. This can be obtained from most opticians at a cost of from 5*s.* to 10*s.*, and ruled up to $\frac{1}{1000}$ of an inch. Zeiss has one at 10*s.* ruled to $\frac{1}{100}$ of a millimetre, *i.e.*, about $\frac{1}{2500}$ of an inch.

Any steady table can be used by the microscopist for working, but it should be looked to that it is not too small, and not polished or varnished on its surface. This surface is best painted a dull, dark colour. The table is so placed that the microscope is about, or somewhat less than, two yards from the window.[a] Any position of the window is good which allows a free outlook. From direct sunlight we protect ourselves by a white roller blind, which is best made of tracing-linen. The dazzling white light which we obtain when the direct sunlight plays upon the blind gives the most favourable conditions for observation with high powers.

The necessary object slides and cover-glasses can be obtained of most opticians. The former are procurable with either ground or unground edges at a cost of about 4*s.* 6*d.* or 3*s.* 6*d.* per gross, respectively. They are three inches long by one broad. The cover-glasses for ordinary observation should be about $\frac{3}{4}$-inch square; but the observer should also have larger ones for specially large objects, and also others somewhat smaller ($\frac{5}{8}$-inch square) which will usually suffice for permanent preparations. If we use powerful objectives, it will be best to obtain these cover-glasses of definite thickness. For the beginner, this is not of special importance; but the more advanced student will find it advisable to procure both object-slides and cover-glasses of a definite thickness.*

* The latter, curiously enough, are difficult to obtain in England, where, nevertheless, they are mostly made, and I get them from P. Stender, in Leipzig, Königstrasse 11. They are 18 mm. square, lettered "C," at a price of 2*s.* 6*d.*

[a] See note on page xxiv.

Further necessary are some plane- and some hollow-ground razors; a fine and a coarse pair of steel forceps; a finely pointed pair of dissecting scissors, for which fine embroidery scissors will serve; a pair of needle-holders, somewhat after the fashion of crochet needle-holders, but so arranged that they will hold the finest needles firmly; English needles from No. 8 upwards, for these holders; some scalpels, some fine painting brushes, a small vice, such as used by watchmakers; some pipettes, glass tubes, and glass rods; watch glasses of various sizes, and glass disks of suitable sizes for covering them; low glass bell-jars (receivers), in order to be able to fit up moist chambers; zinc frames, somewhat as represented in half-size in Fig. 1, on which to place the object-

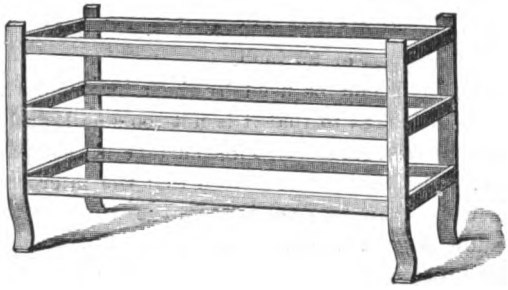

FIG. 1.

slides under the bell-jars;* two bell-jars of suitable height, under which to be able to place respectively the compound and the simple microscope; and lastly, elder-pith. For working, a tumbler of clean spring water is needed; a saucer is useful for dirty slides.[a]

The list of the necessary reagents is to be found at the end of this book. Where the word "alcohol," or "spirit" and not "absolute alcohol," is used, strong methylated spirits can always be understood and is far cheaper.

For the preservation of permanent preparations, many kinds of cabinets and cases are advertised. It is very important to re-

per 100. They are in square boxes, are very light, and come readily by post. Smaller sizes can be obtained, *e.g.* 15 mm. square, lettered "C," at 2*s.* per 100. These are all 0·10 mm. thick. At 0·15 mm. (B) thick these sizes are 2*s.* 3*d.* and 1*s.* 9*d.* respectively.

* Slides can be also left upon these frames to dry, after permanent mounting. If the frames cannot be kept perfectly steady, the slides may wriggle off in time; to prevent this, sheets of paper ½ inch wider than the frame can be bent over them on each stage, and the slides laid across these. By using blotting paper for these sheets, keeping wet, the bell-jar can be converted into a convenient moist chamber for a number of slide cultures at room temperature.

[a] See note on page xxiv.

member that the objects should be kept in a horizontal position, and should be capable of ready supervision.

NOTES TO THE INTRODUCTION.

[1] From the special point of view of botanists: Naegeli und Schwendener, "Das Mikroskop," 2 Edit., 1877; Dippel, "Das Mikroskop," 2 Edit., 1882; and "Grundzüge der allgemeinen Mikroskopie," 1885; Behrens, "Hilsbuch im Botanischen Laboratorium," 1883.

[Carpenter, "The Microscope," 6 Edit., 1881.]

In changing the objective in use from high to low power, or *vice versâ*, much time and inconvenience is spared by the use of a "Nose-piece." This is screwed into the end of the microscope-tube, where the objective is usually placed, and is provided with apertures into which two or more objectives can be screwed; and, by rotating these on a centre, any one can be brought into a line with the tube of the microscope. The best and cheapest are those of Zeiss, for 2 objectives, 20*s.*; for 3 objectives, 27*s.* Both of these are constructed with the "English screw."

[*Note to page* xxii.]

[a] In using the microscope with light taken from a window, light coming from any other direction should be avoided. If a white roller blind is used to pass direct sunlight through, the eyes should be protected in some way from the direct action of the light.

[*Note to page* xxiii.]

[a] For transferring sections from fluid to a slide the camel-hair brushes can be used as described on page 17; or a simple "section lifter" can be made from a straight piece of stout copper wire 4 or 5 inches long, by beating out thin about half an inch or so of one end, cutting the edge smooth with scissors, and then bending the wire at about ½ inch above the broadened part to an angle of about 135°.

I.

USE OF THE MICROSCOPE. STRUCTURE OF STARCH.

MATERIAL WANTED.

Potato, fresh.
Potato starch, air-dry.
Bean meal, air-dry.
East Indian Arrowroot (*Curcuma leucorrhiza*).
West Indian Arrowroot (*Maranta*).
Grains of Wheat.
Grains of Oat.
Stem of the Sun Spurge (*Euphorbia helioscopia*) and of *E. splendens*, fresh. (Other species can replace these if necessary.)

WE will first obtain information about the separate parts of the compound microscope (Fig. 2, p. 2), and for this purpose we select Stand No. VII. A of the manufacture of Zeiss of Jena.* Upon this stand we distinguish the horse-shoe foot (*fs*), the supporting pillar (*sl*), the stage (*ot*), the body or guiding sheath (*fh*), the tube (*t*), the mirror (*s*), and the micrometer screw (*m*).

The mirror-frame (*s*) combines two mirrors, that on the one side plane, on the other side concave. The former we use with weak the latter with strong enlargement, or magnification. The mirror-arm is usually hinged, and sometimes jointed, so that it can be placed obliquely below the stage for oblique illumination. The beginner should always see that the mirror is directly below the aperture in the stage. The stage is pierced in its centre by a circular aperture, which is intended to give passage to the light reflected from the mirror. Under this opening are found the cylinder diaphragms. They are fixed in a carrier, which can

* I retain this, with modifications, as it matters little on what instrument (provided it is of simple construction) the mode of use is described. As this particular instrument is not, and is not likely to be, largely used in England, I have added some supplementary paragraphs on the students' microscopes more commonly in use here. [ED.]

be withdrawn laterally from the stage, and in which can be set diaphragms of various widths, provided with the instrument. With the help of these diaphragms we regulate the illumination according to necessity, a diaphragm with a small aperture allowing little

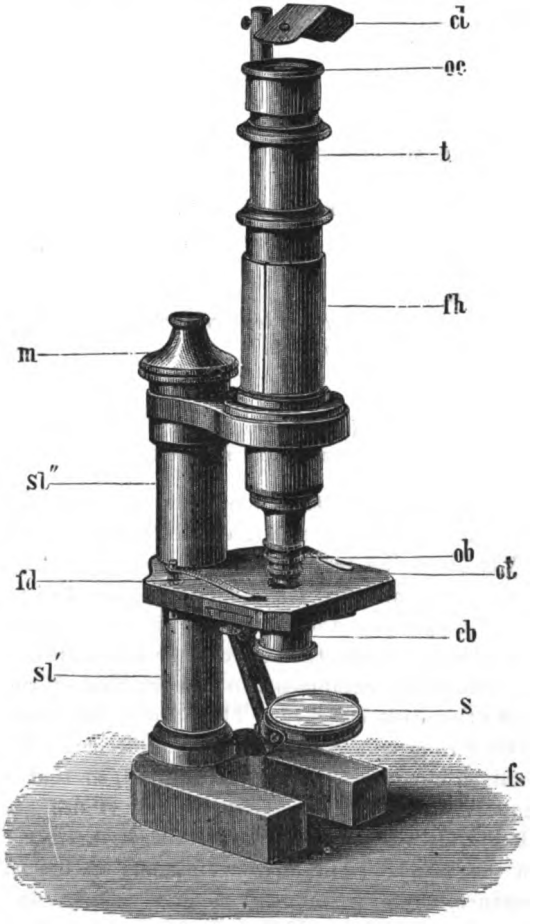

Fig. 2.—Stand No. VII. A of Zeiss, with prism for drawing, *cl*, one-third actual size; *fs*, foot; *sl'*, lower, *sl''*, upper part of the pillar; *ot*, stage; *cb*, cylinder-diaphragms; *fd*, clips; *s*, mirror; *m*, micrometer screw for fine adjustment; *fh*, guiding sheath for *t*, tube; *ob*, objective; *oc*, eye-piece.

light to pass through, and so on in proportion to the size of the opening. Some of the stands of the same maker have, in place of the cylinder-diaphragms, an arched excentrically fixed diaphragm

disk, which is rotated in order to bring different sized apertures into the optical axis of the microscope. This is the kind of diaphragm-wheel which is provided with most of the students' microscopes of English makers. Though not perhaps quite so good, it is more convenient in use. Best of all is what is called an "Iris diaphragm," with which, by simply moving a lever, the size of the aperture can be regulated at pleasure, and with the utmost nicety. Upon the stage are clips (*fd*), which serve to keep the object in position during examination, and are particularly necessary where the instrument is used in the sloping position. If it is possible to do so, we will first remove these. The tube (*t*) is movable in its guiding-sheath (*fh*) which is often lined with cloth to make the movement easier. In larger stands the sheath is wanting, and the tube is raised and lowered by rack and pinion movement. Most of the better makes of English student microscopes have this rack and pinion coarse adjustment, and, for a small sum, most of those which are without it can be provided with it. It is, however, a doubtful advantage for the learner. The chances of accident with its use are perhaps numerically fewer, but when they do occur they are more serious. We withdraw the tube from the sheath and screw into its lower end the weak objective, about B of Zeiss, 3 of Leitz, or half-inch of English make. This will vary with the microscope. As seen in the Introduction, the English microscopes are usually supplied with 1 inch and $\frac{1}{4}$ inch objectives. A much preferable combination would be a $\frac{3}{4}$ and $\frac{1}{6}$ inch. In purchasing it would be easy to arrange this. In microscopes provided with rack and pinion movement, the tube is not withdrawn, but raised sufficiently above the stage to allow the objective to be screwed in. The relative power of the objectives can always be told by the comparative sizes of the front lenses; the weakest power has the largest lens. We now replace the tube in the sheath, and approach the objective so near the stage that it is only removed from it by somewhere over a quarter of an inch. In the upper end of the tube we place the eye-piece, No. 2, or whatever our lowest power eye-piece may be. This likewise may be judged by the size of the glass. English eye-pieces are usually lettered. It is on the whole desirable to use for general purposes the lower (weaker) eye-piece of the instrument of any maker. The drawing prism found over the eye-piece in the figure we pass over for the present. We place our instrument opposite to a window, and at a distance of

about, or somewhat under, a couple of yards from it. While we now look down through the eye-piece, we change with the fingers the inclination of the mirror until the field of view of the microscope appears to us bright and equally illuminated. In this we have to take care that the mirror is not (as, for example, it looks in the figure) pushed forwards or laterally out of the axis of the instrument, as we propose to observe by direct (not oblique) illumination. On the other hand, in this stand and in most English stands, we can, according as required by the strength of the light, slide the mirror on its bearer upwards or downwards in the optical axis of the instrument, thereby approaching it more nearly to the stage, or removing it therefrom. The majority of English microscopes, instead of being supported on a single pillar (*sl'*) as in Fig. 2, have the body swung between two uprights, between which it is hinged, much as in Fig. 81, in Chap. XXI. hereafter. This gives greater possibilities from the point of view of illumination, has other advantages, and in the large "English stands," properly so called, is a necessity for observation. The learner is, however, strongly urged to learn to work with the instrument erect. The clips, then, are unnecessary for ordinary work. With a sloping stage, some appliance for keeping the object-slide in position is a necessity.

An object-slide is now wiped clean, and upon it, by means of a glass rod, a drop of spring water is placed.

We will now commence with the investigation of a potato tuber. We cut this through with a pocket knife, and transfer a little of the sap which exudes from the cut surface into the drop of water by means of the same knife. We then cover the drop with a cover-glass. This must also have been previously cleaned with special care. It is done best flat between the fingers with pieces of old linen.* The cover-glass must be laid on as carefully as possible, so as to exclude air from underneath it. For laying on, it can be held between the index-finger above, and a

* This operation is not so simple as it seems. If the cover-glasses are thin they are very readily broken. The method I have found least destructive for learners is to hold the cover-glass by its edges between thumb and index-finger of one hand. Having slightly damped the same fingers of the other hand make a fold in a piece of silk, with the damp fingers flat above and below it, slip the glass horizontally between, and gently rub the silk-covered fingers to and fro over its two surfaces. The silk will cling to the slightly damp fingers, and the process becomes easy. Some use little pads between which the cover-glass is placed, and the pads then moved about over its surfaces. [ED.]

needle underneath it. By gradually withdrawing the latter when the cover touches the drop of water, it is lowered into its place. If the drop is of proper size, no water will flow out from the side of the cover-glass. The size of the drop has usually to be calculated from the point of view of (1) the size of the cover-glass, and (2) the thickness of the preparation to be covered. Here the latter does not come into the calculation. If water does flow out it can be removed with blotting-paper, or it is better to make a second preparation, as in this case most of the grains which we wish to observe will be sucked out by the blotting-paper.

We now place our preparation on the stage of the microscope, so that the object lies over the centre of the stage-aperture. In order to focus correctly, we first slide the tube, carefully controlling its motion, so far downwards that it almost touches the object. Then, while at the same time looking through the eye-piece, we move the tube as slowly as possible upwards. This movement is best combined with a twisting of the tube inside the body-sheath. Soon the moment arrives when the previously invisible object begins to show itself in the form of small grains. If, on the other hand, we find we have removed the objective (object-glass) more than about $\frac{3}{4}$-inch from the object-slide, without having caught sight of the grains, these either do not lie in the field of view of the microscope, or we have raised the tube too quickly, and so overlooked the rapidly appearing and equally rapidly disappearing object. We must not then attempt by sliding the tube downwards to find the object, as thereby we should run into the danger of breaking the cover-glass, injuring the object, and destroying the objective (object-glass); instead, we a second time slide the carefully controlled tube so far downwards that it almost touches the object-slide, and begin anew to raise the tube, more slowly than before, and at the same time looking through the eye-piece. If this also should not realize our purpose, it is to be assumed that the object does not lie in the field of view, and must be looked for again after altering the position of the object-slide. After a short time it will happen in all cases that the grains appear in the field of view, and we then discontinue sliding the tube, *i.e.*, what we call the coarse adjustment, and attain the fine adjustment which now is wanted by the aid of the micrometer-screw (*m*, Fig. 2). This we turn in one direction, or, in case the object thereby is made more indistinct, in the opposite direction. The adjustment (**focussing**) is

perfect when the figure appears as sharp as possible. In our example of a microscope stand (Fig. 2), the micrometer-screw is at the upper end of the pillar (*sl''*); but it can be variously placed according to the make of the instrument. In instruments of larger size, as in many English students' instruments, the coarse adjustment is not effected by hand and sliding tube, but by rackwork and pinion.

After we have determined by slight magnification the existence of small grains in the field of view of the microscope, and have noted, for subsequent use, the distance of this weak objective from the object, *i.e.*, its focal or working distance, we leave the object-slide unmoved upon the stage, but withdraw the tube from its guiding sheath, unscrew the weak objective and screw in a stronger one, in no case as yet however an immersion objective, but rather about D of Zeiss, No. 7 of Leitz, or a $\frac{1}{4}$ or $\frac{1}{6}$ inch of the English makers. We then replace the tube in its sheath, and push it down so far that once more the objective almost touches the cover glass. We again endeavour to catch sight of the object by raising the tube in its sheath. With a stronger magnification it must however be withdrawn far more slowly than with the weaker. As the preparation has lain unmoved upon the stage we know it to be certain that the object will be found in the field of view of the microscope. When the grains have become visible with the coarse adjustment, we complete the fine focussing (adjustment) with the micrometer screw. We shall find that the working or focal distance of the stronger objective is considerably less than that of the weaker one, and always less in proportion as the objective is stronger.

We now begin the actual observation. The learner should accustom himself, so far as his two eyes are equally good, to observe with his left eye. The right eye is thus kept free and can be used in drawing while he continues to observe with the left eye. Many of the drawing prisms and appliances for the microscope are moreover constructed for the left eye (as shown in Fig. 2); and those who work with the right eye should intimate it on ordering such drawing prisms. The learner should also keep open the eye which is not in use. At first the surrounding objects which are figured on the retina of the eye will disturb him; but he will soon overcome the difficulty of concentrating all his attention on the eye in observation, and entirely suspending the activity of the other.

We readily recognise that the colourless bodies which occupy the field of view of the microscope are solid and show lamination. They are **starch grains**. We slowly move the object-slide here and there, in order to find a place where the grains do not lie too closely, because it is easier here to fix attention on a single grain. We select for persevering observation a grain which shows the lamination with special clearness. As the movement of the object-slide under the microscope appears to be reversed, we shall at first find some difficulty when we wish to place a selected grain in the centre of the field of view; and we shall have as quickly as possible to accustom ourselves to sufficiently control the slight movements upon which it depends. If we have found a single specially favourable grain, we magnify it still more by now removing the weak eye-piece and replacing it by a stronger. Hold the tube of the microscope firmly while you do this, or the focussing may be altered, and the objective perhaps run down on the cover-glass. With perfect objectives the figure always remains good, though in all cases the light diminishes. We endeavour by improving the position of the mirror as far as possible to obviate this inconvenience.

Now and then, after focussing the preparation, or after moving it, it will happen that the figure has lost in clearness. In all probability this is because fluid from the preparation has got upon the under lens of the objective. This will happen especially easily when too large a quantity of fluid has been used, and has run out from under the edge of the cover-glass. We must then withdraw the tube from its sheath, and after having proved the supposition, wipe the front lens of the objective with a clean and often-washed piece of linen rag, or, still better, rub it with a freshly broken surface of a piece of elder pith.

The starch grains of the potato tuber attain a comparatively considerable size. They are excentrically constructed, as their organic middle point (*c*, in *A*, Fig. 3) is not the geometrical centre, but lies considerably nearer to one end. The layers appear variously sharp (*A*); between those more strongly marked can be seen others more weakly marked. Towards the surface of the grain the layering becomes indistinct. For optical reasons, and on account of its smaller density, the organic centre, or nucleus, appears rosy coloured. It shows up most clearly when it is hollowed. It then shows as a rosy point, as a line, cross, or star with dark outlines. The layers immediately surround-

ing the nucleus are developed concentrically, soon however the excentricity has influence, in that the layers diminish in thickness towards one end of the grain, so as partly in this direction to run out into a wedge. At this more weakly developed end of the grain, which we can distinguish as the anterior end, the layering, on account of the small distance from the surface, is indistinct. The individual grains vary considerably in size, and moreover they deviate from one another in outer form to a not unimportant extent, and show the layering with various sharpness. Between the starch grains in most preparations will be found rounded bodies, which with median focussing show a small, round, bright centre and a broad, dark margin; this last is black at its inner edge, dark grey outwardly, and interrupted by a clear ring. These structures are **air-bubbles** enclosed in the fluid under observation. Their appearance under the microscope is so characteristic that, once known, they can scarcely ever be confused with other appearances. The rays of light which pass out of the denser medium into the air-bubble are, with the exception of the central ones, so strongly refracted, that they cannot get into the objective, and hence the broad dark edge and the comparatively small clear middle. If, by turning the micrometer screw, the tube is lowered, so that the under part of the air-bubble comes into view, the sharpness and brightness of the middle disk increases; it diminishes at the same time in size, while the breadth of the surrounding dark ring increases. If the screw is moved in the opposite direction, in order to focus upon the upper part of the air-bubble, the middle disk enlarges, but losing somewhat in brightness; a grey ring of differing brightness arises around it; the surrounding edge becomes simultaneously narrower.

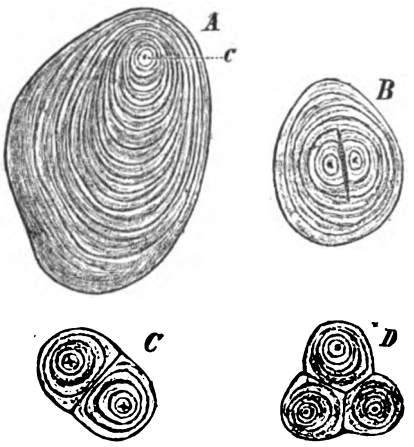

FIG. 3. — Starch grains from a potato tuber, *A* simple grain, *B* half-compound grain, *C* and *D* entirely compound grains. *c* the nucleus (× 540).

If the observer has selected a beautifully laminated starch grain, it should now be drawn. The greatest possible stress is decidedly laid upon drawing in microscopical observation. With the help of it we in general first learn to see quickly. Then the peculiarities of the figure first become present to the mind of the observer, when he concentrates his attention upon it for the purpose of reproduction. Drawing therefore protects from transient superficial observation, enforces a penetrating, thorough study of the figure, and sharpens more than any other means our power of observation. The learner should first endeavour to represent the object by free-hand drawing. So much drawing ability as is necessary for this he may perhaps possess, but can however readily obtain by practice the necessary facility. The object should not be drawn too small, even if the observer believes he sees it very small. A correct opinion on the size of the object in the field of view of the microscope is only obtained after long practice, and it is better at first that the learner should draw the object too large, in order conveniently to include in his figure all the details of the object. No less important is it to provide the individual parts of the figure with suitable distinguishing names ("terms"), and to note the name of the plant, the object, and the most important results of the observation.

The starch-grains of the potato are somewhat flattened, as can be easily demonstrated if, during the observation, you push carefully with a needle against the edge of the cover-glass, and so set the grains rolling. Upon the smallest grains the layering is usually but little recognisable.

Besides the simple grains (as in *A*, Fig. 3) will be found also, after some search, semi-compound grains (as in *B*). These grains enclose two, rarely more, organic nuclei (or centres). Each nucleus is surrounded by a number of its own layers, both together by a smaller or larger number of common layers. Not infrequently the two inner complexes of layers are separated by a cleft, extending to the common layers (*B*). The number of layers peculiar to the individual grains, as well as of those common, varies according to circumstances.

The completely compound grain, which is found far more commonly than those half-compound, consists of two (*C*), less frequently of three (*D*), rarely of more than three component grains. As a distinction from the semi-compound grains, the common layers are wanting in those quite compound. In the

line connecting the nuclei of the component grains the layers are most strongly developed. The component grains therefore turn their posterior ends towards one another, their anterior ends away from one another. The line of separation between two component grains often broadens internally into a cleft.

For comparison we now put up a preparation of potato starch which has been preserved in an air-dry state. We proceed in this quite similarly to the preparation of the first object, and transfer a trace of the meal into a drop of water. As the object-slides may differ in thickness, it is advisable to raise the tube of the microscope prior to placing under it the new preparation. This is not of course necessary in using the low powers.

The first preparation, as it will be again required later, we place in a large moist chamber. This moist chamber consists of a deep plate and a glass bell-shade with knob. On the plate stands the zinc frame, which we discussed and figured in the Introduction (Fig. 1); so much water is also poured into the plate till the bell-shade has its lower edge quite immersed in it. The preparation is laid upon the frame. But first we assure ourselves that the drop of water under the cover-glass of the preparation is not already partially dry. If this should have happened, we place at the edge of the cover-glass, so that it shall be sucked in, a new drop of water. We also mark the object-slide, and best with a coloured crayon which writes directly on the glass.

Upon examination of the new preparation we shall find that the lamination of the air-dry starch is at least as sharp as that of the fresh. This preparation also we place in the moist chamber.

We further make a preparation of air-dry bean flour (*Phaseolus vulgaris*). The grains (Fig. 4), examined in water, appear circular or oval; they are a little flattened; a certain medium size predominates. The lamination is very clear and very uniform; the lamellæ show almost equal thickness. The structure is concentric. The nucleus of grains examined in water is hollowed, more isodiametric in the rounded, elongated in the oval forms. From the nuclear hollow extend radial clefts, which cut through the layers at right angles, and, thinning off, reach almost to the periphery of the grain.

Fig. 4.—Starch-grains from the cotyledons of *Phaseolus vulgaris* (× 540).

We lay a trace of this bean-meal, in similar manner, in a drop of glycerine instead of in water. In this fluid the starch-grains seem on the average smaller; of lamination not a trace can be recognised; the inner hollow and the clefts are wanting. These are formed under the influence of water, in which the bean-starch swells somewhat.

The starch of the East Indian arrowroot (*Curcuma leucorrhiza*) is otherwise constructed. We put up a preparation of the commercial starch, which is usually not difficult to obtain. Genuine East-Indian arrowroot shows in its grains a very excentric structure (Fig. 5*A*), at the anterior end strongly tapering, beautifully and regularly layered, and very flat. Often a considerable number of grains cling together by their flat sides, and, viewed from the edge, appear like rolls of coins (*B*). The size and form of the grains varies not inconsiderably.

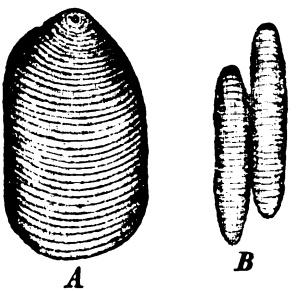

FIG. 5.—Starch-grains from the commercial East-Indian arrowroot (from the rhizome of *Curcuma leucorrhiza*). *A*, seen from the surface; *B*, several grains clinging to one another, seen from the edges (× 540).

The West-Indian arrowroot, also called in short Arrowroot, from the rhizome of *Maranta*, especially of *Maranta arundinacea*, is easy to obtain in shops, but gives however, from the point of view of its structure, much less interest than the East-Indian arrowroot. Observed in water, the grains show great similarity to the starch-grains of the potato; only they are usually less clearly, and, in exchange, more uniformly layered; somewhat more rounded; on the whole smaller; also more uniform in their size. At the position of the nucleus is usually found a cleft in the form of a wide open V.

Wheat meal shows the layering very badly; as relatively the most favourable, we choose the starch-grains of *Triticum durum* for observation. We halve the grain of wheat with the pocket-knife, and scrape off a little substance from the cut surface, and put it in the drop on the object-slide. The large starch-grains are circular, discoidly flattened, and regularly laminated (Fig. 6 *A*), but the layers are usually hard to

FIG. 6.—Wheat-meal from *Triticum durum*. *A*, a large, *B*, small grains.

see. In many grains they will, nevertheless, be recognised, as well as the central nucleus. [a] As a characteristic appearance will be found in the preparation, besides the large starch-grains, and almost without transition sizes, small grains, with clear rosy nucleus, but without recognisable lamination. A number of such grains are represented at *B*. In many preparations compound grains are not altogether rare; in most they are sought for in vain, as they have fallen into their component grains.

A *B*

FIG. 7.—Starch from *Avena sativa*. *A*, a compound grain; *B*, its component grains (× 540).

The starch-grains of the oat (*Avena sativa*) we take as the best, inasmuch as we halve an oat-grain and take a little for observation under water. The compound grains here are met with in great beauty, such as is represented in the adjoining figure. The size of these compound grains varies, and proportionally also the number of the component grains entering into its structure. The Fig. 7 *A* represents such a compound grain of medium size. The individual component grains appear polygonal, separated from one another by clearer looking boundary lines. Between the great grains are seen small ones, down to such as consist of but two component grains; lastly also quite simple ones; besides also numerous angular grains (*B*) which arise from the large compound grains broken down in making the preparation. A medium size, somewhere about our Fig. *A*, is met with by far most commonly amongst the compound grains. The lamination in this object is not visible, the nucleus is only exceptionally indicated.

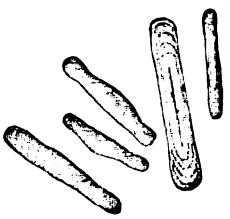

FIG. 8.—Starch-grains from the latex of *Euphorbia helioscopia* (× 540).

Of quite peculiar appearance are the starch-grains in the latex (milk) of the Euphorbiaceæ. A piece of the stem of a spurge is cut off, and the cut surface is plunged in the drop of water which is ready upon the object-slide. The latex which flows out from the cut surface mingles with the drop. We can select for example the universally distributed *Euphorbia helioscopia* [sun-spurge] for our investigation. In the latex, which appears distributed in small drops, like an emulsion, in water, we shall see isolated, small, rod-like bodies (Fig. 8). These are the starch-grains in

[a] See note on page 15.

question. They appear pretty strongly refractive; a lamination is visible only in the most favourable cases; sometimes a longitudinal cleft is recognisable in the interior of the grain. The size of the rods is somewhat variable, many of them are a little swollen in the middle. Much more beautifully formed grains of this kind are possessed by the tropical Euphorbiaceæ. We choose for this examination *Euphorbia splendens*, so commonly grown in plant houses, and make the preparation in the same way as stated above. The starch grains which now put in an appearance (Fig. 9) have the form of bones. In the same latex will be found others shaped like rods, and still others with greatly enlarged ends, like dumb-bells; they appear more or less swollen at both ends, are somewhat longer than those of our native forms, and in the swollen parts permit something of the lamination to be recognised. Very commonly we see a colourless vesicle adhering to the sides of the grain (*A*), the walls of which, however, are referable, not to the substance of the starch-grain, but the plasma mass adhering to it. It must strike the observer that the small latex globules distributed in the water are in tremulous motion. This is the so-called Brown's molecular movement [the "Brownian movement"], which we can therefore take this opportunity of learning to know, and which, not a phenomenon of life, is referable perhaps to fine streams in the fluid carrying with them the minute bodies.

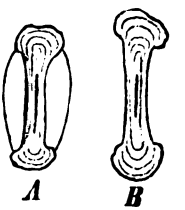

FIG. 9.—Starch-grains from the latex of *Euphorbia splendens*. One of the grains has a vesicle attached laterally (× 540).

After getting this information on the form and structure of the starch-grains, we will produce some reactions upon them, and study directly, under the microscope, the result of the action. We take first a preparation of potato-starch again out of the moist chamber. After focussing we place a drop of a solution of iodine (iodine-water, alcohol-iodine, or tincture of iodine, or potassium-iodide iodine) at the edge of the cover-glass. In using the reagent we must take special care that the drop does not run upon the cover-glass and thence upon the objective. If a drop comes upon the cover-glass, let it be immediately sucked off with blotting paper. If the reagent reaches the objective, plunge the lower lens of this latter in pure water, and clean it afterwards with the pieces of linen rag already recommended.

In order to see the action of the iodine solution directly, await its penetration to a spot previously selected, this spot, however, being chosen not too far from that part of the edge of the cover-glass at which the reagent is placed, and follow by movement of the object-slide the progress of the action. We see, immediately the influence of the iodine solution begins to make itself felt, the starch-grains stain bright blue, and rapidly ever darker till they are black-blue. At the first moment of the action the lamination shows up clearly, only immediately to disappear in the grain when it becomes opaque. With potassium-iodide iodine solution, in case this is added in considerable quantity, the action produces quickly a dark-brown coloration of the grain. Similarly dry starch-grains, which are exposed to the action of iodine vapour, become deep dark-brown. If we add water to such a preparation the brown passes rapidly into blue. If the action of the reagent should not proceed rapidly enough under the cover-glass, it can be readily accelerated by fragments of blotting paper placed at the opposite end of the cover-glass. [b]

We should stain with iodine solution the rod, etc., shaped grains of the *Euphorbia* also, in order to demonstrate that, in spite of their variable form and of their scarcely noticeable lamination, these bodies are true starch-grains.

Let us further study the phenomena of the **swelling** of starch-grains under the influence of potash (potassium hydrate). First we again take potato-starch, and await the entrance of the reagent, placed at the edge of the cover-glass. The action of this must take place quite gradually, if it is to be instructive. We then notice, at the first moment of the action, that the lamination stands out more clearly, quickly, however, to disappear, while the grain increases in size. During this enlargement, which proceeds with more or less regularity, the nucleus of the starch-grain hollows considerably, upon which the wall of the weaker side, therefore towards the anterior end of the grain, sinks into the hollow. Later on the regularity of the phenomena disappears altogether, and the grain enlarges to a mass as clear as glass, of considerable volume, the limits of which are scarcely distinguishable.

Finally, we can endeavour by warming the preparation to cause the starch to swell, a treatment such as indeed is in use in the preparation of paste. The preparation is warmed over a spirit or gas flame, without allowing it to boil, and taking care to replace

[b] See note on page 15.

the evaporated water by fresh. If in warming a temperature of about 70° C [approx. 160 F] is reached, the grains will be found to be swollen just as in treatment with potash. [If it is wished to determine accurately the temperature at which swelling ensues, the warming of the preparation must be effected upon a special table which can be heated, and its temperature registered. Such a table by Ranvier,[3] can be specially recommended.]

With this we close our first Lesson.* Before we put the microscope on one side we carefully clean, in the manner before described, the objectives and eye-glasses, together with any other pieces of apparatus that we have used. We withdraw the microscope tube from its sheath in order to rub it, and also the interior of the sheath, with a rough towel. Instead of again replacing the microscope in its cabinet, we prefer to place it under a glass bell-jar, which latter, in order to protect the instrument as much as possible from dust, can have its lower edge covered with felt.

NOTES TO CHAPTER I.

[1] Compare herewith Nægeli, *Die Stärkekörner*, in *Pflanzenphys. Untersuchungen*, Heft 2; E. Strasburger, *Bau und Wachsthum der Zellhäute*, p. 107, where the further literature will be found.

[2] Ranvier, *Traité d'Histologie*, p. 41. 1875.

[*Note to page* 12.]

[a] A frequent appearance upon these grains, and capable of recognition with but low magnification, is the presence of a beautifully regular network, usually upon only a small portion of the surface of the grain. The network is formed by ridges arranged into a net, and similarly the meshes are occasioned by shallow depressions of the surface of the grain.

[*Note to page* 14.]

[b] The most beautiful violet-blue coloration of the starch-grains is obtained, however, when a scale of iodine is laid amongst the starch-grains in the drop of fluid under observation. The coloration commences immediately in the vicinity of the scale.

* The chapters in the original are called "tasks," or "lessons."

II.

ALEURONE-GRAINS, PROTEIN CRYSTALS, FAT OIL, MOUNTING OF PERMANENT PREPARATIONS, USE OF THE SIMPLE MICROSCOPE.

MATERIAL WANTED.

Dried Peas.
Grains of Wheat.
Seeds of Lupine (*Lupinus*).
Seeds of Castor-oil (*Ricinus communis*).
Brazil Nuts (*Bertholletia excelsa*).

WE examine, first of all, the **Pea** (*Pisum sativum*). A ripe seed is halved by a sharp pocket-knife, and in such a manner that the two cotyledons (seed-leaves) are cut across. Take then from the cut surface a thin cross section with a sharp, hollow-ground razor. On the subject of section-cutting with the razor the following points can be noted:—1. The cut surface is to be moistened before cutting the section, most commonly with water, though in this case with glycerine, since the preparation suffers from water, and we shall observe it in glycerine. 2. The first section is not to be used, as here the tissue would be too much injured by the pocket-knife. 3. In such resistant tissues as that of the pea only very small and exceedingly thin sections ought to be taken, as the edge of the razor would be very easily notched. If the razor has gone too deeply into the tissue, and it is seen that the resistance to its progress increases, it is better to withdraw the razor, instead of forcing it to the end of its cut. 4. Unless the investigation requires it, it is advisable not to commence the section with the outer surface of the object, but rather to lay the razor on the cut surface, as thus a far firmer support is obtained in order to get a thin section. 5. In order to get a really good section, that is one in which the individual elements of the tissue are not torn, the razor must not merely be pressed with its edge against the object, but at the same time drawn across it. It is well, therefore, in order to cut as freely as possible, to accustom yourself not to rest the

thumb of the cutting hand upon the other hand. Instead of this, both hands can with advantage be rested against the breast, because thereby lateral movement of the cutting hand is not hindered. The back of the razor should be supported on the index finger of the hand supporting the object. 6. As it is difficult to hold so small an object as a half pea, especially when it is also so hard, sufficiently firmly between the fingers, it is recommended to use for the purpose the small hand-vice described in the Introduction. The half pea is therefore fixed sufficiently deeply in this. 7. It is not advisable to be satisfied with a single section, but to take a considerable number, in order to make choice of the best.

The section selected should be observed in glycerine, either concentrated or diluted with one-third distilled water. Pure water is not available for this, because it quickly sets up appearances of disorganization in the ground substance of the cells. The transfer of the section from the razor to the glass slide is best made with a fine camel-hair brush. The section is removed by pressing the brush upon it and sliding it off from the blade. If it adheres to a sufficiently broad surface of the brush, rolling up ("curling") of the section will be prevented; curling occurs very easily, on the other hand, if the section is taken directly by its edge with the tweezers and so transferred. The section adhering to the brush is immersed flat in the drop on the glass slide, and the brush withdrawn laterally with a simultaneous twisting movement. If it is desired to turn a section over when on the object-slide, the brush can be pressed down on the object-slide so that it is in contact with the edge of the section, and then begin to turn it over away from the section. In this way the section will be very easily drawn upon the upper surface of the brush, and can then be turned over with it. Other similar tricks will soon be acquired in practice. After every time of use the brush must be most carefully washed in water.

Examine the section of pea with a strong magnifying power. It proves to be a **tissue** composed of rounded **cells**. At the places where three such cells adjoin one another a triangular **intercellular space** (*i*) filled with air, is present. The air appears black, like the edge of the air bubbles previously described; here it naturally must show the form of the space, since it fills it. The **wall** of the cells (*m*) is pretty thick. In the adjoining figure the three middle cells are completely, the surrounding ones only partially, represented. In each cell can be seen the large starch-grains (*am*), and

with some care also the small grains (*al*) which lie between them. These grains are, for their part, imbedded in a very finely granular **ground-substance** (*p*). From thin parts of the section many a starch grain will have fallen out; a hollow of similar form and size in the granular mass will indicate these places. The small grains are **Aleurone** or **Protein-grains**[1]; they lie in the ground substance of the cell. If we run iodine solution into the preparation, the coloration which ensues gives us immediate information as to the individual constituents of the cells. The drop of iodine solution is placed at the edge of the cover-glass; as, however, the iodine solution diffuses very slowly in the glycerine, and it is not our present purpose to study the progress of the reaction, we accelerate it a little by slightly raising the edge of the cover-glass with a needle, and so permit the mixture of the iodine with the glycerine. A second needle placed at the same time against the opposite edge of the cover-glass prevents it from slipping. The starch-grains colour blue to violet; the aleurone-grains and the ground-substance yellow. By the use of potassium-iodide iodine the coloration of the aleurone-grains and ground-substance becomes very intense; but the starch-grains are at the same time over-coloured, and appear then black-brown. If sections of pea are laid in a drop of alcoholic borax-carmine solution, in a very short time the ground-substance, and also almost simultaneously the aleurone grains, colours dark-red; the starch-grains remain colourless. The reaction becomes especially striking if, after the section is thoroughly soaked in the carmine solution, this is replaced by dilute glycerine or by water. This is done by sucking out the carmine solution by a piece of blotting-paper placed at the edge of the cover-glass, while at the same time the water or dilute glycerine is run in under the opposite edge. If a section is placed in Millon's reagent, the starch-grains swell very strongly, and

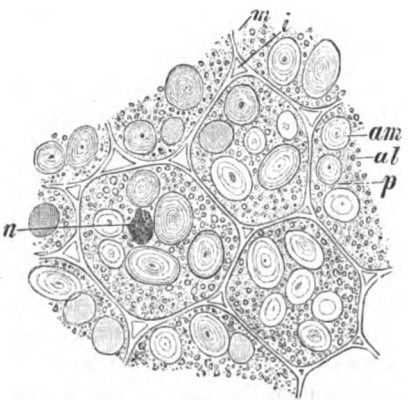

FIG. 10.—From the cotyledons of the Pea. *m*, cell wall; *i*, intercellular space; *am*, starch; *al*, aleurone grains; *p*, ground substance; *n*, nucleus.

become unrecognisable; aleurone and ground-substance are immediately disorganized; the disorganized mass however, after some time, takes on a characteristic brick-red colour. If still another section is laid in aceticized methyl-green, after a short time there appears in each cell, between the other constituents, a greenish-blue spot of rather indefinite outline. This spot is the **Nucleus** (*n*). The other constituents of the cell have not stained; the starch-grains are just a little swollen (they show radial clefts, which are wanting in glycerine), and the aleurone-grains also have increased in size, and appear as if porous or even hollow. We recognise therefore in aceticized methyl-green a reagent which in the present case recommends itself as a special staining material for the nucleus. Simultaneously, it is true, the cell-walls also stain, but this does not injure the value of aceticized methyl-green as a reagent for nucleus staining. The cell-walls appear of a beautiful bright blue colour, and, as the result of this, are traced out in the glycerine preparations much more readily than before. The intercellular spaces also stand out more sharply.

In the yellow-brown iodine reaction, the accumulation of colour materials, and the brick-red from Millon's reagent, we have learned to know the most important means whereby to recognise under the microscope *albuminous bodies*, for to these belong aleurone-grains as well as protoplasm and nucleus. Protoplasm, as will be seen again later, shows these reactions first when it is dead; in this case death results from the action of the reagents. The substance of the nucleus shows a specially strong affinity for the colour materials.

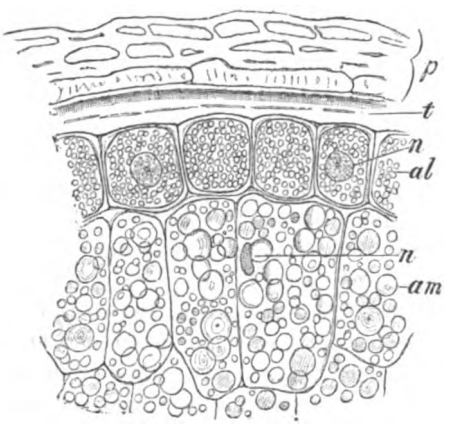

FIG. 11.—Cross section through a grain of wheat (*Triticum vulgare*). *p*, pericarp of fruit; *t*, testa of seed. In the endosperm cells succeeding to these: *al*, aleurone-grains; *am*, starch-grains; *n*, nucleus (× 240).

A grain of wheat (*Triticum vulgare*) can be recommended as a second object of investigation. The grain is first halved (across) with a pocket knife, then one half fixed in a small vice in order to

have sections taken from it. This time it is desirable so to take the sections that a piece of the skin also is represented on them. In cutting, moisten the cutting surface with glycerine, and observe the object in the same fluid (Fig. 11). Under the skin, formed of cells pressed closely together and dead (*p*), which represents the combined skin of the fruit (pericarp) and of the seed (testa), lies a layer of rectangular cells, which are thickly filled with small aleurone-grains (*al*). The aleurone-grains are embedded in a finely granular ground-substance. Then follow elongated, less regular cells, which contain large and small starch-grains. This is not difficult to determine with suitable reactions.

We will now "mount" a successful section of the wheat-grain, and by this means learn how to put up a permanent preparation or, to use the common phrase, how to permanently "mount" a preparation. We will employ first the simplest method of preparation, which is here so much the more desirable, as it gives a very favourable result: we enclose the section in glycerine-jelly. Place upon the glass slide so much of this jelly-like substance as we believe will suffice to form a drop. Then warm the glass slide slowly over the flame of a spirit-lamp, till the jelly has become fluid. The section is then laid in the drop, and a cover-glass placed over it. It is advisable first to warm the cover-glass a little, as otherwise air-bubbles will easily remain in the preparation, and for similar reasons it is desirable not to place the cover-glass on quite horizontally, but with a slight lateral movement. If, in spite of this, air-bubbles are enclosed, the glass slide can be warmed a little, and by careful raising of the cover-glass endeavour to bring the air-bubbles to one side. If the air-bubbles are not troublesome, the task of removing them can be given up. If several sections are placed in the same drop they should be uniformly dispersed in it. Truly it often happens that, in laying the cover-glass upon them, the sections come into contact with one another, and even lie upon one another. If the cover-glass is raised on one side to secure order, the contrary to this is often produced. Another comparatively simple method is therefore employed. By warming the glass slide, make the drop as fluid as possible, and then, without lifting the cover-glass, pass in a hair from one side. With this hair seek out the object to be rectified, an operation which usually tends to succeed. Before covering with the cover-glass it is, above all, necessary to make sure that no particles whatever of dust have found access to the drop of

glycerine-jelly; any such should be removed with the needle. As these manipulations can only be carried on with a suitable magnification, this is at the same time the moment to learn the use of the simple microscope in connection with the methods of preparation under the compound microscope.

I assume in the first place that the observer has at his disposal a small dissecting microscope (compare Introduction, p. viii.), either as Fig. 12, or some other of like construction. Over the stage (*ot*)

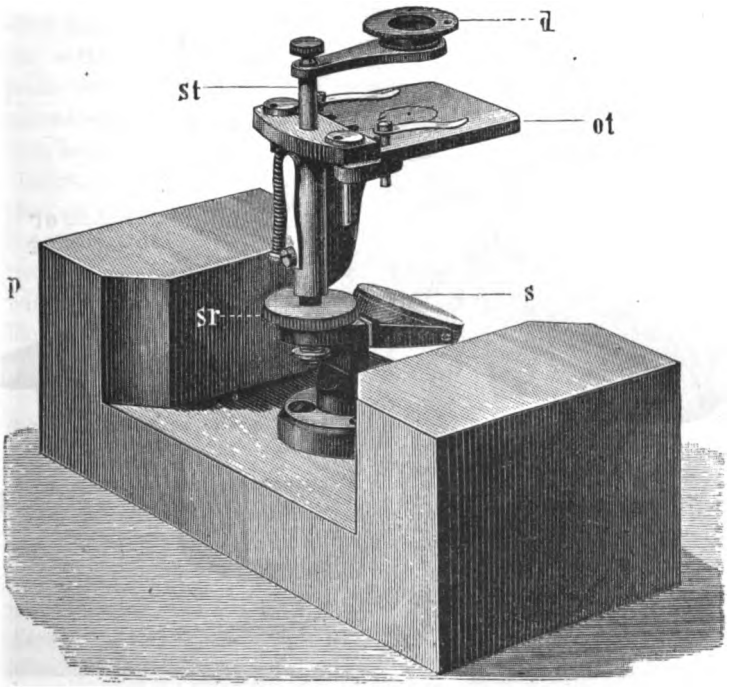

FIG. 12.—Small dissecting microscope of Zeiss, on foot, two-thirds natural size. *Ot*, stage *d*, lens, sheathing toothed support for lens-arm; *sr*, screw for fine adjustment; *s*, mirror; *p*, wooden supports for hands in dissecting, etc.

of this small dissecting microscope (Fig. 12) is placed a lens (*d*), borne on a horizontal arm. The horizontal arm is fixed to a steel upright (*st*), which can be moved up and down inside a tube. By this movement is brought about the coarse adjustment. The fine adjustment is effected on the other hand by turning the screw (*sr*). The instrument is screwed into a dissecting foot, the high ends of which (*p*) serve as resting-places for the hands in the

processes of preparation or dissection. The instrument is provided with two, or with three lenses, magnifying 15, 30, and 60 diameters, and it is an advantage also to have lenses magnifying five and ten fold.

The larger dissecting microscope of Zeiss (comp. Introduction),

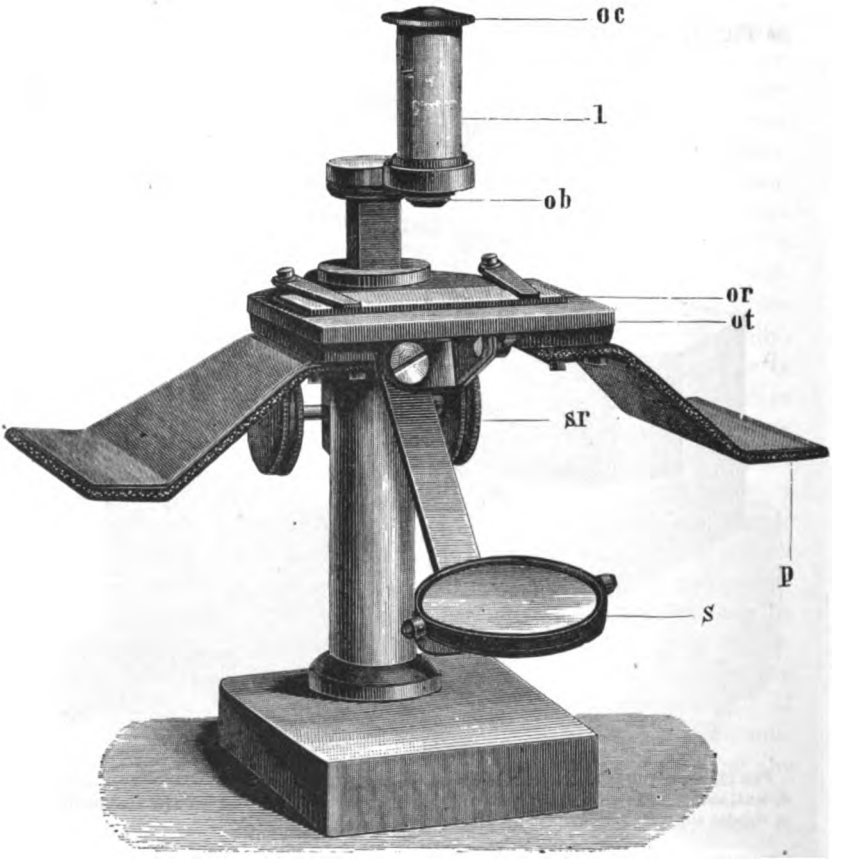

FIG. 13.—Large dissecting microscope (Zeiss), half natural size. *ot*, stage; *p*, wings as arm rests; *sr*, screw head for adjustment; *l*, system of lenses, of which *ob* is the objective, *oc* the eye-piece. Upon the stage is an object-slide fixed with the clips.

or other of similar construction, has also a system of lenses (*l*, Fig. 13), consisting of three achromatic lenses, which can be combined into an objective (*ob*), a tube, and an achromatic eye-piece. In order to work with slighter magnification, the objective can be

used alone as a lens, the eye-piece, together with the tube, being unscrewed. The three lenses of the objective can also be unscrewed from one another, and the upper lens alone can be used, the two upper, or the three simultaneously. Magnification of 15, 20, and 30 diameters can be thus obtained. The adjustment is completed by turning the screw-head (*sr*). On both sides of the stage (*ot*) "wings" (*p*) are fixed, to serve as hand supports in dissection.*

In order to prepare or to dissect with the compound microscope, what is called an "erecting eye-piece" can be used in the place of the ordinary eye-piece of the microscope. This "erecting" eye-piece reverses the image of the object; and as, in a compound microscope, the image is normally upside down, it is thus rectified. It is, however, quite possible, though to a beginner very difficult, to dissect, etc., with the ordinary compound microscope. With practice one comes to realize that every movement is reversed, and to govern the movements accordingly. The low powers can then be freely used for dissection and preparation. In dissection, etc., with the compound microscope it is of advantage to have two blocks of wood of suitable size, which can be placed on either side of the stage, and will serve to support the hands.†

Whichever of these instruments is used for preparation, we first lay the preparation on its stage, that we may free it from any foreign bodies which may happen to be present. For this purpose the lowest magnification that is at our disposal is used. This, in the larger microscope for preparation, of Zeiss (Fig. 13), is fifteen diameters. The distance of the object from the lens would then be about $1\frac{1}{4}$ inch. With this instrument, even with the strongest magnification, viz., 100 diam., this distance is more than $\frac{1}{3}$ inch After proper adjustment of the mirror (*s*) and of the image, take in each hand a needle fixed in a holder (see Introduction), steady the hands on the rests, bring the points of the needles into the axis of the instrument, and endeavour to see both simultaneously in the field of view of the microscope. This will soon be success-

* I have retained the above descriptions intact, as they illustrate pretty fully the structure of dissecting microscopes in general. For an account of other instruments by English makers, see the Introduction. In choosing an instrument I would specially urge the importance of stable arm-rests, as in the above Fig. 12. An instrument satisfying all the requirements of even more than the beginner ought not to cost more than about 30*s*. [ED.]

† This remark equally applies to those forms of simple (or dissecting) microscopes which are unprovided with rests. [ED.]

fully accomplished, and then by means of a few experimental attempts learn how to make the necessary slight movements with the needles. This easy problem of removing the foreign bodies out of the preparation with the points of needles will soon be completed to our satisfaction, whereupon we proceed to lay the cover-glass upon the drop of fluid. If this in the meantime shall have become too viscid, it can be again warmed before being covered.

The glycerine-jelly preparations need no further enclosing, are therefore prepared in the simplest possible way; and as most vegetable objects, even stained ones, preserve very well in glycerine-jelly, we can recommend this method in preference to others.

The preparation must then be labelled, preferably at both ends of the glass slide, with gummed circles or squares of paper, upon which must be written at least the name of the plant, the nature of the object, the direction of the section, if it be one, the medium in which it is preserved, any staining material used, and the date. If it is desired to keep the preparation slides stacked on the top of one another, then they must be protected from contact by card-board labels in the place of those of paper. The cardboard labels should be cut the breadth of the glass slide, by about $\frac{5}{8}$-inch in the other direction. On these the information, as above, can be written. The card-labels are best fixed on with "Crystal Palace Cement," or other similar medium, or they can be fastened with Canada balsam dissolved in turpentine.[a] If it is necessary to fasten them with gum, it is best to cover each end of the slide first with a strip of gummed paper, the ends of which shall fold over and overlap under the slide, and fasten the card label on these; otherwise the label would easily spring away from the slide.

Take now the seed of the white Lupine (*Lupinus albus*), or other allied species. Once more halve the seed across, and take sections from the moistened cut surface. Sections observed in water show in the cells rounded aleurone-grains with vacuoles. In order to see the grains in their natural form they must be observed in glycerine. The grains then appear at first refractive, angular, gradually forming in their interior a fine network, granular. Lying closely together they fill up the cell; a small quantity of ground-substance lies between them, more ground substance can be observed against the walls of the cells. The walls of the cells are very strongly thickened and pitted, a structure which we shall, however, study later on a more favourable object. In iodine-glycerine the grains take a beautiful golden-yellow colour.

[a] See note on page 27.

In the next place remove the shell-like testa from the seed of the castor oil plant (*Ricinus communis*), cut it through across, and make preparations just as above from it. The tissue of the endosperm is capital material to cut; it contains very much fat oil, and need not be moistened. The sections can be observed in water, the disturbing effects of which, by removal of oil, come but gradually into operation. The aleurone-grains, imbedded in a ground-substance very rich in fat (Fig. 14, A), enclose in their interior usually one, but sometimes two or more, **Protein-crystals** [or so-called **crystalloids**], and usually a single rounded body, the **Globoid,** which is of inorganic composition, the combination of double phosphoric acid with lime and magnesia. With longer action of water the ground-substance in which the aleurone-grains lie is disorganized; great masses of oil collect around and on the object. These cling partly to the object and the glass, and have an irregular form, partly lie free, and then are globular. They are mostly clouded with numerous vacuoles. If the microscope is adjusted so as to show an optical section of such an **oil drop,** it appears bright grey, and is surrounded by a narrow black limiting zone. If the tube of the microscope is lowered, the dark ring disappears; the disk appears somewhat more brightly surrounded. If the tube is raised, the dark zone, which in the mid-position of the tube is narrow, becomes broader. Oil-drops show, therefore, reverse appearances to those which have been previously observed in air-bubbles. Air refracts light less, oil more strongly, than water; hence their opposite relations. These relations should be noted for future observation. Bodies which are less refractive than the medium in which they are observed, have an inner brighter part which, with deeper focussing, is so much the smaller, an outer darker part which is so much the broader; with more strongly refractive bodies these relations are exactly reversed.

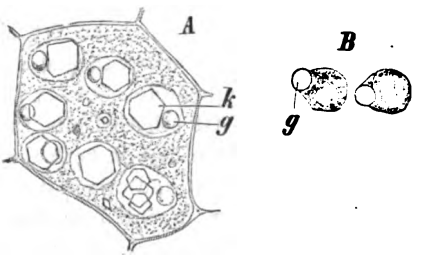

FIG. 14.—From the endosperm of *Ricinus communis*. *A*, a cell of the endosperm with its contents, viewed in water; *B*, single aleurone-grains seen in olive oil; *g*, the globoid; *k*, the protein-crystal (×540).

If we run absolute alcohol under the cover-glass of the preparation of *Ricinus*, which is at present in water, the preparation will

"clear" somewhat; and simultaneously the protein-crystals in the aleurone-grains come out very sharply. They are now so clearly defined that this method of manipulation is recommended in order to study their form,—hemihedral tetrahedra of the regular system. [2] After longer action of the alcohol, the oil-drops disappear more and more, as castor oil, in contradistinction to other fat oils, is miscible with absolute alcohol. Now make another preparation of *Ricinus* seed, lay it on the glass-slide in a drop of glacial acetic acid, and cover it with a cover-glass. The protein-crystals swell and disappear in the aleurone-grains. These latter increase considerably in volume, the globoids also enlarge, and show up very clearly in each aleurone-grain. Drops of fat are, however, not visible, because castor oil, again acting as an exception, mixes with glacial acetic acid. Otherwise absolute alcohol and glacial acetic acid, because normally they either not at all or but slightly dissolve **fat oils**, while on the other hand they are solvents of **ethereal oils**, are the best reagents for the purpose of distinguishing between these two classes of oil under the microscope. Of ethereal oils, the terpene dissolve somewhat less easily than the others in both the above reagents. Chloroform and ether dissolve fat and ethereal oils equally.

To a preparation mounted in water run in alcanna (alkanet) tincture diluted with water. The fat masses soon accumulate colour and stain reddish brown, a reaction which ethereal oils and also resin alike show.

Logwood (Hæmatoxylin) added in small quantity to a preparation in glycerine, stains the protein-crystals a beautiful violet. In olive oil the protein-crystals are not visible; the whole grain on the other hand appears a strongly refractive, rounded body, at one of the ends of which the globoid simulates the appearance of a vacuole (Fig. 14, B). The protein-crystals come out very beautifully if the section is laid in a drop of 1% osmic acid; they gradually take on a brownish tint. By the same reagent the oil is slowly blackened, a peculiarity which fat oils have in common with ethereal oils; this reaction is, however, not characteristic, as many other organic substances become black in osmic acid.

Protein-crystals of extraordinary beauty, which show readily all the characteristic protein reactions, are to be found in the endosperm of the seeds of *Bertholletia excelsa*, the well-known "Brazil nut." In this also the sections are exceedingly easy to obtain. If to a preparation laid in water is added absolute alcohol,

the protein-crystals come out very sharply. The fat oil is not touched to any extent by the alcohol. It remains unchanged also in glacial acetic acid, while the protein-crystals are immediately dissolved. In 1% osmic acid the crystals become very distinct. These crystals are so large, that their form can be made out even by comparatively smaller magnification. Near the crystal lies a globoid, this latter being here always in the form of an irregular aggregation of rounded bodies. The ground-substance is very rich in fat, and with 1% osmic acid becomes everywhere quite black. The granular contents of the aleurone-grain also take on quickly a dark coloration, while the crystals themselves colour slowly yellow. The crystals are optically uniaxial, and belong to the hexagonal system.

NOTES TO CHAPTER II.

[1] Compare Pfeffer, *Jahrb. für wiss. Botanik*, viii. p. 429, where the other literature will be found.

[2] Schimper, *Unters. ü. d. Proteïnkrystalle d. Pflanzen.* Inaugural Dissertation, Strassburg, 1878.

[*Note to page* 24.]

[a] Card-labels for object slides. Any one of the numerous forms of glass-cement, such as coaguline, mend-all, etc., will answer this purpose. A quantity of slides can be prepared at one time. The card-labels are cemented on both ends of the slide, and these are tied into bundles to dry under pressure. The cards recommended at p. 34, for drawing, cut into admirable labels. This method is very economical for keeping preparations, as all those of a similar kind can be tied into a bundle, with a plain slide over the uppermost, through which its label will be visible. Preparations preserved in glycerine do not then necessarily need to be closed with Canada Balsam, provided the preparation is thin, so that little glycerine is used. [Ed.]

CHAPTER III.

MOVEMENTS OF PROTOPLASM; NUCLEUS. DRAWING WITH THE CAMERA, ETC.; CALCULATION OF MAGNIFICATION.

Material Wanted.

Flowers of *Tradescantia* (best *T. virginica*). Fresh. Or, very young shoot of a *Cucurbita* (gourd, pumpkin, cucumber, vegetable marrow, etc.).
Young roots of the Frog-bit (*Hydrocharis morsus-rana*) or of *Trianea bogotensis*. Quite fresh.
Strong, oldish, leaves of *Vallisneria spiralis*. Fresh.
Young parts of *Nitella*. Fresh.

We will first study now the phenomena of the movement of living protoplasm, and select as one of the most favourable objects for this purpose the hairs on the staminal filaments of *Tradescantia* (the Spider-wort). *Tradescantia virginica*, and other closely-allied species, are cultivated in every botanical garden, and flower from May or June till late into autumn. The long violet hairs in every flower will at once strike the eye. For observation, select hairs out of a flower which is either just opening or has just opened. The preparation is made by seizing a tuft of hairs at the base with the forceps; remove them and lay them in water. Or the whole filament can be placed under a cover glass if the anther is previously removed. In this last case the masses of air clinging amongst the hairs will give trouble, and it takes some pains to remove them. This is best effected by means of a fine camel-hair brush, with which the hairs are brushed over from below upwards, the tuft being at the same time held firmly at the base. After this the cover glass is laid on. Most of the hairs will not have suffered, provided the air has been removed with sufficient carefulness.

The hairs in question are formed of numerous cells, swollen into a barrel form, and arranged into an unbranched row. At the points of constriction lie the partition walls which separate the

individual cells from one another. Each cell (Fig. 15) shows a thin continuous lining layer ["peripheral layer"] of **protoplasm**, and is traversed in the interior by numerous thinner and thicker protoplasmic strands. Suspended within these strands is to be found the **nucleus**, surrounded by an enveloping layer of protoplasm. (Shown somewhat below the middle in the figure.) The cell cavity in which the nucleus is suspended, and which is traversed by the protoplasmic strands, is filled by a violet-coloured **cell sap**. It is the **vacuole**. The protoplasm consists in a colourless, viscous, semi-fluid substance, which is distinguished by the name of **Hyaloplasm** [*i.e.*, clear plasma], and which contains numerous minute granules, called by the name of **Microsomata**, or **Microsomes**. Besides these there can also be seen in the protoplasm, in greater or less number, somewhat larger, highly refractive bodies, which appear somewhat bluish in colour, and which will be designated by the terms **Starch-formers**, **Starch-builders**, or **Leucoplasts**. If we focus the object-glass from the peripheral protoplasm inwards, it will be seen that this is not in movement as a whole, but that rather the fine, net-like, anastomosing, protoplasmic strands flow into and away from it. In the protoplasmic threads which surround the nucleus the movement is especially strong. These streams are of various thickness, they anastomose laterally with one another more or less frequently, and the nucleus manifestly furnishes a central point for them. Most of the threads end in the plasma layer surrounding the nucleus. The current in a single strand moves often only in one direction; often, however, it can be seen that even in very thin strands or threads there are two currents in opposite directions. The movement is recognisable by the microsomes and leucoplasts borne in the clear basal hyaloplasm. With continued observation it will be seen that the strands slowly change their thickness, arrangement, and other conformation. New branches of the system can be seen to arise, others can become constantly thinner in the middle, finally break through and withdraw into other strands. Thus by degrees the figure changes. The nucleus is almost globular, in many cases oval or somewhat flattened. With

FIG. 15.—A cell from the hair on the filament of *Tradescantia virginica* (× 240).

the strongest magnification which is at our command it appears finely punctate, and in it can be readily distinguished some larger granules (**Nucleoli**). Often two nuclei lie close together in such a cell, because the original nucleus has divided. The nucleus is towed about hither and thither by the plasma strands, and thus slowly changes its position in the cell. In order to prove this, take rapidly a sketch of the cell, and compare this with the arrangement of the nucleus and the currents after the lapse of some time. Such a sketch can only be accurately taken by means of a drawing prism, and it alone has definite value for later comparison. We will, therefore, endeavour here to become acquainted with the use of the drawing prism.

The camera lucida of Abbe recommended first of all in the Introduction, which is represented in ideal longitudinal section

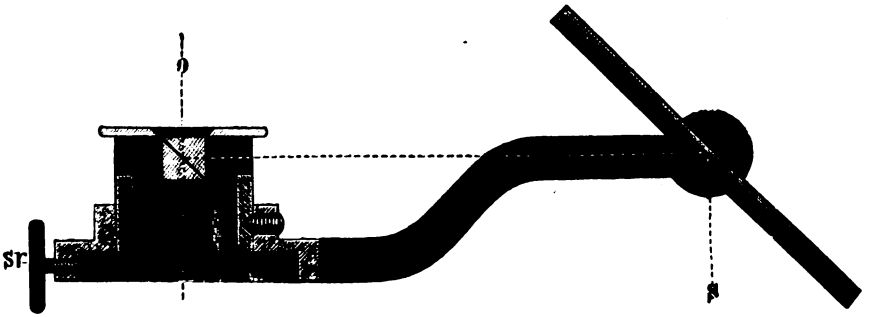

FIG. 16.—Camera lucida of Abbe, nat. size. Ideal longitudinal section. The course of the rays of light indicated by the dotted lines; *o*, the position of the eye; *s*, the direction of the surface for drawing upon; *sr*, clamping screw.

in Fig. 16, is, as shown in the figure, placed upon the eye-piece and fastened with the clamping screw shown at its side (*sr*). It is best to remove the eye-piece before screwing on the camera, as in the performance of this manipulation upon the microscope there is the danger that the tube may be pressed down, and the preparation crushed. When the eye-piece with the camera is placed in the tube, then, in case we use the microscope with the left eye, the mirror of the camera should be placed in front; but in case of use of the right eye, to the right hand, and inclined about 45°, in the manner shown in the figure. If now we look through the camera in the direction of the eye-piece, we see once more the figure of the object in the field of view of the microscope. Now place in front of, or to the right side of, the microscope a

horizontal drawing desk, this being thereabouts the height of the stage of the microscope. Lay a sheet of drawing-paper upon this desk, and rest the point of a lead pencil against it. If the point of the pencil is found under the mirror in the direction of *s*, this must now be visible in the field of view of the microscope at the same time with the figure of the object. The point of the pencil is, however, made visible by double reflection, the first time in the large mirror, the second time in the silvered surface of a small prism in the point of sight of the eye-piece (compare the figure) while the microscopic figure comes directly to the eye through a small opening in the silvering of this prism. If the surface of the drawing desk does not lie in the distinct visual distance of the observer, the point of the pencil will be seen indistinctly, and the drawing desk must be raised, or, though seldom, be made lower. We test the necessary height by means of books laid one upon the other. The microscopic figure is only well visible on the drawing surface when a definite relation of brightness exists between the two. Dimming of the drawing surface can be produced by the aid of smoked glasses, which are made to turn on the camera. If the arrangement is perfect you can draw with the lead pencil the outline of the object as if drawing it in the field of view of the microscope.

The second camera mentioned in the Introduction is seen in Fig. 2, set upon the microscope in the position for drawing. This camera has the advantage that it can always be kept on the instrument, and with some practice will perform yeoman's service. It consists of two prisms, inclined to one another, in a common setting. The rays coming from the pencil take, after double reflection inside the prisms, a course parallel to the axis of the microscope, and thus coincide with the rays coming direct from the object. The camera is placed in the inclination represented in the figure, and so placed that its anterior edge, visible through the opening in the setting, approximately bisects the "pupil" of the emerging rays of the microscope, *i.e.*, the bright circular disk which we notice when we look perpendicularly into the eye-piece from a short distance, such as $1\frac{1}{2}$ inch, above it. If, then, on moving the head to one side we do not see the "pupil" notably displaced towards the edge of the prism, this latter stands also at the right height. We draw upon a sloping drawing desk, which is placed in front of the microscope. If, after some attempts, we have found the point of the lead pencil upon the

drawing paper, we can now follow with it the outlines of the object. If the object is not to be distorted in drawing, the drawing desk must have the correct inclination. In order to determine this, we use a method of procedure which quickly leads us to our end. We draw the circular outline of the field of view upon the paper with the aid of our camera, and obtain thus, if the inclination of the drawing desk is correct, likewise a circle *i.e.*, the cross measurements of the figure from side to side and from top to bottom of the sloped surface will be like; if, on the other hand, we have an ellipse, the slope of the drawing desk is not correct, and must be varied until a circle is produced. Or, we set in position, and always with strong magnification, the stage micrometer recommended in the Introduction, *i.e.*, a millimeter divided into 100 parts, engraved upon an object slide. We now turn the stage micrometer around through 90°, so that the engraved lines shall run from side to side, and succeed one another fore and aft. In case the too small size of the stage does not permit such a position of the stage micrometer, we must change the position of the microscope 90°. The turning of the microscope naturally renders necessary a change of the direction of the mirror. If our instrument is provided with a "concentric rotating stage," or similar appliance, then it is only necessary to turn this; such a stage is very useful for drawing, as it enables us to place the object in the desired position. If we have given the micrometer its proper position, we draw, with the help of the camera, its lines upon the paper on the drawing desk. The lines follow one another up the slope of the desk. We shall succeed, without much practice, in reproducing it exactly; but, as the lines have a definite thickness, it is necessary that we should represent always a definite edge of the line. The inclination of the drawing desk is correct when the distance apart of the lines remains the same at all heights. If this distance increases upwards on the desk, the desk must be made steeper; if it sinks, it must be placed in a less-inclined position. As, for the rest, small mistakes are not excluded from our measuring scale, it is necessary to represent several parts of it in the same way. In this way we shall find that our desk should have a slope of about 25°. Having once found the correct slope, it is well to have a desk made with its two supporting sides of the correct heights.

This figure, when we have obtained the correct inclination of the drawing desk, can be, at the same time, used in order to

calculate the magnification of the drawing *i.e.*, the magnifying power of the *system*, or combination of objective and eye-piece, in use. We know already that the lines which we have drawn are 0·01 millimeter (*i.e.*, approximately $\frac{1}{250}$ inch) removed from one another. If we find that now they lie 2·4 mm. (*i.e.*, nearly $\frac{1}{10}$ inch), we know that the drawing is enlarged 240 times. This method is also the simplest and best for measuring the size of the microscopical object. If we have, that is, attained the necessary accuracy in drawing, in order to reproduce even slight variations in size with fidelity, and if we know the definite enlargement of the object which we have drawn at exactly the same distance, it needs only to divide the size of the drawing by the known enlargement to get the actual size of the object. If, *e.g.*, one cell of the hair of *Tradescantia* appears, with 240 times enlargement of its figure, to be 9 mm. broad, this indicates an actual breadth of $\frac{9}{240}$ mm., *i.e.*, of 0·0375 mm. This method gives in the simplest way such a close result, that in our investigations we can limit ourselves to it.

Various other contrivances have been introduced for the purpose of aids to drawing. Some of these, like the Wollaston Camera Lucida, require the body of the instrument to be placed horizontally, and the instrument as a whole to be raised on a pedestal. This can of course only be used with instruments which admit of this position; and for working purposes it is, besides, objectionable in several ways. A very cheap form for use thus is Dr. Beale's neutral-tint reflector, which fixes on the eyepiece, making with its glass an angle of 45°. The student, when he chooses a camera or drawing-prism, should always select one for use with the instrument in the vertical position; and, as he may not improbably obtain one from a maker who is not the maker of his instrument, he should always send the eyepiece of the latter, so that the fittings of the camera may be adjusted to the size of this. Zeiss's camera is adjusted for eyepieces of the continental size, a size much used by English makers for their smaller instruments. Of whatever camera is chosen the method of adjustment upon the eyepiece must be learned from the maker (though usually very easy to find out for one's self); the rules laid down above for learning how to draw are equally applicable to all of them. Lastly, the quality of the drawing depends on two factors: 1st, the accuracy of the observer, and 2nd, the skill of the draughtsman.

Now turn once more to the cell of the *Tradescantia* hair, and

endeavour with one or another drawing apparatus to make a figure of it. As in all drawing apparatus which are not strictly cameras, some manipulation for the regulation of the light is needed, so we must endeavour, either by shading the drawing surface, or by changing the position of the mirror, to obtain thereabouts similar brightness for the surface of the drawing and the field of the microscope. For drawing, it is best to use stiff, smooth drawing-cards * and black-lead pencils. In order that they shall not be effaced, finished drawings can be washed over with very dilute gum-water.

Take in this way a sketch of the entire outline of the cell, of the protoplasmic streams and the nucleus, and compare it after some hours, to see whether the form and circumstances now correspond. As already indicated, we shall most probably find that the distribution of the streams has altered, and that the nucleus has changed its position in the cell.

In order to determine that in their streaming the cells are independent of one another, and that the cell-wall does not influence the movement, allow a neutral but water-withdrawing fluid to act upon the cell. Under the cover-glass add to the drop of water a little concentrated sugar solution, or, better still, glycerine. Before long the reagent begins to withdraw the water of the cell-sap, and there results a decided contraction of the protoplasmic sac [*i.e.*, the lining layer] into the cell. This withdraws from particular places of the cell-wall. This contraction of the protoplasmic body of the cell under the influence of dehydrating (*i.e.*, water-extracting) media is distinguished by the name **Plasmolysis.** It can be then observed, that so long as the contraction does not become too strong, the streaming of the protoplasm still goes on, even in those parts where it has withdrawn from the cell-wall. Soon, indeed, all movement in the cell is arrested. Yet in most cases to set it going again it suffices to wash out the water-extracting reagent by means of water. To this end water should be run under one edge of the cover-glass, while the fluid under the cover-glass is sucked out from the other edge by blotting-paper. The protoplasmic sac then again tends to expand and reach the cell-wall. It not infrequently happens that during the contraction single pieces of the protoplasm separate themselves from the cell-body, and remain lying against the cell-wall as rounded

* Such, of excellent quality and surface, are Goodall's thin Bristol Boards. ED.]

balls. These balls can also be retaken into the expanding protoplasmic sac.

It is easy to determine that during the contraction of the contents, observed as above, the colour-material does not diffuse through the living protoplasmic sac, and that the coloration of the cell-sap becomes proportionally more intense. [a] The appearances in dead cells are quite otherwise. For example, allow absolute alcohol to act upon the hairs. The protoplasm is immediately killed, and now the peculiar property of coagulated protoplasmic masses, to accumulate colour materials, is set in action. The protoplasm withdraws from the cell-sap the violet colour, and this soon appears quite limpid, while the cell-plasm and the nucleus stain deep violet. The violet colour can now pass through the protoplasmic sac, and diffuse in the surrounding fluid. [b]

If *Tradescantia* should not be at the disposal of the observer, other hairs can be substituted for it. A very favourable object is provided by the hairs which grow upon the youngest shoots of the genus *Cucurbita* [gourd, pumpkin, vegetable marrow, cucumber, etc.]. The preparation is made by removing these hairs at their base by a razor, and bringing them into a drop of water on a slide. The stronger hairs are multicellular at the base, and pass into a tapering cell-row; others bear multicellular heads. The protoplasmic network in the cells is finely developed; it contains microsomes, and, though but sparely, large, green-coloured **Chlorophyll-grains.** The nucleus is large, suspended by the threads; it has a brightly shining nucleolus, and is carried about hither and thither in the cell.

A very peculiar object is provided by the root-hairs of *Hydrocharis morsus-ranæ* [the Frogbit]. For the investigation are selected fresh young roots with stiff hairs. The hairs are visible to the naked eye. Cut off an entire root-point, and quickly place it on the slide in a sufficient quantity of water. The cover-glass is laid on in the usual way, and the largest cover-glass at our disposal should always be chosen. In this way the preparation is made, although it is true that, owing to the not inconsiderable thickness of the object, all parts will not be accessible with stronger magnification, because the object-glass will come into contact beforehand with the cover-glass. These hair-cells are very long and tubular, and, like all root-hairs, unicellular. The protoplasm, which it richly contains, is in active movement, but there are here, not numerously divided thin streams, formed into

[a] [b] See notes on page 37*a*.

a network, but a single strong stream, moving round in the protoplasm lining the wall. This kind of movement is called **Rotation,** to distinguish it from the other kind, or **Circulation.** This stream, thus returning to the same place, presents the appearance of a broad, slightly spirally turned band, which, if projected upon a plane would form a very elongated figure **8.** The movement must not, however, be represented as if the band, as a connected whole, were turned around inside the cell, for, in fact, the neighbouring parts during the movement are continually changing their reciprocal position. The two streams going in opposite directions are, however, not in immediate juxtaposition, but are separated by a narrow band of protoplasm which is at rest. This "neutral band" is reduced to a very thin layer of protoplasm.[c]

The leaves of *Vallisneria spiralis* furnish very instructive preparations for illustrating rotation of protoplasm. This plant is cultivated in all botanical gardens, and very commonly also in aquaria in rooms in houses. For investigation a strong leaf is selected, and a section taken from the lower part of it. For this purpose it answers best to lay the long, narrow leaf across the index finger, and to hold it down on both sides with the thumb and middle finger. The surface section is taken by moving the razor parallel to the long axis of the leaf. The aim should be to obtain a plate or "lamella" of tissue about half the thickness of the leaf [but if the section should at first sight appear too thick, parts of it which are sufficiently thin for the purpose will probably be found]. This lamella is laid on a slide, epidermis downwards, in a drop of water. Air clinging to it may make some parts of the section useless, but others will always be found which admit of undisturbed observation. The streaming always goes on for some time before it is discontinued; it can be best followed in the wide elongated cells which form the interior of the leaf. At low room temperatures the movement is sluggish, but it can be hastened by slight warming of the microscope slide. The stream circles around the entire cell, without, in most cases, to any extent deviating from its direction parallel to the long axis. The "neutral band" is pretty broad. The stream carries with it green-coloured chlorophyll grains and the nucleus. The latter is flattened into the form of a disk. From time to time it comes into sight, but as a rule it is concealed by chlorophyll-grains. Not infrequently it sticks at a turning point, then the accompanying

[c] See note on page 37*a*.

chlorophyll-grains also halt with it, till, an instant later, all again are drawn into the stream. The direction of the streaming changes from cell to cell without any regularity. If glycerine or sugar solution is permitted to act upon the section, the protoplasmic sac can be seen to withdraw from the cell-wall, and the continuance of the streaming at the first moment of contraction can be readily made out.

The strongest protoplasmic currents known in vegetable cells are met with in the *Characeæ* (Stoneworts). We must, however, take the genus *Nitella*, for the genus *Chara* has completely invested, and therefore opaque, internodes, while the internodes are specially suited for the investigation. For observation we select the younger members of the plant, and can state immediately that the rotating layer of protoplasm possesses a very distinct thickness. The outer layer of protoplasm immediately lining the cell-wall, in which the chlorophyll-grains lie, is motionless. The motionless layer is here, therefore, comparatively thick, while it is in general so thin as to escape observation. For in all earlier investigated objects also an outermost denser layer of protoplasm, the so-called primordial utricle (or **Ectoplasm**) takes no part in the movement. An obliquely mounting stripe or band on the wall of *Nitella* is free from chlorophyll grains; it attracts the eye by its lighter coloration. This band, wanting in chlorophyll, marks the neutral band in the protoplasmic stream. It repeats here the like appearance with the root hairs of *Hydrocharis*, where we found the neutral band of the protoplasmic layer likewise extremely reduced. The internodal cells of *Characeæ* are multinuclear, the protoplasmic current carries with it numerous elongated nuclei, which it is true show up as brighter spots only in the most favourable cases. If the piece ofthe plant is laid for 12 to 24 hours in 1% solution of chromic acid, they can often be very readily seen, and their peculiar rod-like, curved, and horse-shoe forms made out. Not to be confused with these nuclei are the rounded balls which are seen carried around in the stream in larger or smaller number. These appear either smooth or with a spinous surface; as to their significance there is uncertainty. Simultaneously with their forward movement, these balls are turned upon their axis, which shows that the rapidity of the stream is greatest by the stationary chlorophyll-containing outer layer of plasma, and gradually diminishes towards the cell-cavity.

NOTES TO CHAPTER III.

Tradescantia virginica is a quite hardy perennial, and can be grown in any garden. It dies down in winter. Flowering period, June to August.—[Ed.]

[*Notes to page* 35.]

[a] It is a universal rule that, so long as the cell lives, the colouring matters dissolved in the cell-sap cannot diffuse through the denser layer by which the protoplasm is limited externally.

[b] The phenomena which are produced when the hairs of *Tradescantia* are laid in a drop of 10 per cent. solution of nitrate of potash, and then taken under observation, are specially interesting. Most of the cells, it is true, show ordinary plasmolysis; but cells will also often be found in which a barely perceptible contraction of the plasmic body has ensued, whereas the cell-cavity, filled with its violet cell-sap, has collected together as an independent structure. In such cases the cell-plasma is quickly killed, with the exception of the layer which surrounds the cell-cavity. This layer is therefore distinguished by its comparative independence and greater resisting power. At length the cell-cavity forms one or several vacuoles, filled with dark, violet-coloured cell-sap, lying in the disorganised cell-plasma. That the plasmic layer surrounding the cell-sap remains living is proved by its opposing the passage of the colouring matter. If a little watery eosin has been added to the solution of nitrate of potash, the dying plasma, together with the nucleus, is immediately stained red. (Compare H. de Vries, "Plasmolytische Studien ü. d. Wand der Vacuolen," in *Jahrb. f. Wiss. Bot.*, Bd. xvi., p. 465.)

[*Note to page* 36.]

[c] In winter, or in other times in the year, when *Hydrocharis* cannot be obtained, it can be replaced by *Trianea bogotensis*, a South American Hydrocharidean, cultivated in most botanical gardens. The form of the root-hairs agrees entirely with that in *Hydrocharis*, as also does the rotation in the fully-developed root-hairs. The young root-hairs, on the other hand, show active circulation, with abundantly branched, frequently changing, currents. In general, the streams in the lining-layer of protoplasm move towards the tip of the hair, and make their way from thence as threads, which traverse the cell-cavity. Between this circulation, and the definitely restricted rotation, all stages of transition can be observed. The cell-plasma contains microsomes, besides pretty numerous, strongly refractive, globular bodies, partly, perhaps, leucoplasts, and vacuoles of different sizes. In the cell-sap are to be seen more or less small stellate agglomerations, probably of calcium oxalate, which are driven about hither and thither by the action of the plasmic streams.

CHAPTER IV.

CHROMATOPHORES. COLOURED CELL-SAP.

MATERIAL WANTED.

Funaria hygrometrica, or Prothallia of a Fern. The former (moss) very commonly grows on ground which has been charred, or limestone walls, etc.; the latter, on pots and walls of fern-houses.
Flowers of the garden "Nasturtium" (*Tropæolum majus*).
Flowers of the Snapdragon (*Antirrhinum majus*).
Flowers of the Periwinkle (*Vinca major* or *minor*), or other blue flower.
Flowers of the Larkspur (*Delphinium consolida*).
Flowers of *Adonis flammeus*.
Root of Carrot (*Daucus Carota*).
Autumnal leaves of Virginian creeper (*Ampelopsis hederacea*).
Autumnal leaves of *Ginkgo biloba* (*Salisburia adiantifolia*); or Maple.
Flowers of the Mullein (*Verbascum nigrum*).
Rhizome of *Iris germanica*.

[All required fresh.]

WE have already had an opportunity in several objects of obtaining an insight into the structure and enclosures of the chlorophyll-grains or bodies; nevertheless, we will give our attention somewhat specially to these structures. We select for this purpose a very widely distributed moss, which is distinguished by very fine, large, lenticular **Chlorophyll-bodies**, and of which the leaves, unilamellar with the exception of the midrib, permit observation without further preparation. This moss is *Funaria hygrometrica*. Numerous chlorophyll-bodies of considerable size are to be seen in every cell; in plants which are exposed to diffused daylight they are contiguous only to the free cell-walls; that is, to those which form the upper and under surface of the leaf.* From this they present their broad side to the observer. That they are narrower in profile we see in the separate grains which underlie the side walls. All stages of division of the chlorophyll-bodies are easy to find, and often associated in the same cell (Fig. 17). The resting grains appear quite circular; they then become elliptic, afterwards constricted in the middle so as to be shaped like a figure of eight,

* [This is commonly known as the position of *Epistrophe*.]

and finally completely divided across. The two young grains remain for some time still in contact. The starch-enclosures of the chlorophyll-bodies are, according to their varying sizes, in many leaves easy, in others difficult to see. They are, however, always clearly distinguishable when the chlorophyll-bodies get out of an opened cell into the surrounding water, and are there disorganized. To this end we cut a leaf with a sharp pair of scissors into several pieces. The starch-grains, liberated from the disorganized chlorophyll-bodies, augment in size, and are identified as such with iodine. On the other hand an entire uninjured chlorophyll-body is coloured brown with iodine, always as a result of the combined blue coloration of the starch-enclosures, the yellowish brown coloration of the protoplasmic ground-substance, and the green of the chlorophyll. In order to obtain favourable iodine coloration of the uninjured chlorophyll-bodies, we take for investigation leaves which have lain some time in alcohol, and are thereby decolorized. The chlorophyll-bodies now appear colourless; their starch-enclosures take on the coloration by gradual entrance of the iodine solution, earlier than the protoplasmic body. The iodine reaction is still more noticeable if the preparation is previously treated with potash, which causes the starch-grains to swell.[1] This last method also permits the smallest quantity of starch in the chlorophyll-bodies to be recognised. This succeeds so much the more surely with fresh grains if they are treated with a solution of five parts of chloral hydrate in two parts of water[2] to which a little iodine solution has been added on the object-slide. The chlorophyll is dissolved, so that in a few minutes the leaf appears colourless; simultaneously the chlorophyll-body swells, and also the starch-grains which it contains, and these last come out clearly with their blue colour. Leaves decolorized with alcohol show also very beautifully, with the same treatment, the blue-stained starch-grains in the chlorophyll-bodies, while these last are not coloured. After the chlorophyll-bodies have been decolorized by alcohol they can be stained also very well with very dilute watery solution of Methyl violet or of Gentiana violet. The cell membranes also are always coloured hereby, but the chlorophyll-bodies are darker, and therefore stand out more sharply.

Fig. 17.—Chlorophyll-bodies from the leaf of *Funaria hygrometrica*, resting and in division.

With stronger magnification the living chlorophyll-bodies of the

leaf of *Funaria* appear to be finely punctate, and thus betray a network structure.

The same results as with the leaves of *Funaria* are obtained with *Fern prothallia*, so that the two objects can mutually replace one another. Prothallia are always readily to be found in plant houses in which ferns are cultivated; any species equally available for this investigation.

In order to become acquainted with colour-bodies (**Chromatophores**) of other coloration, let us turn next to *Tropæolum majus* [the so-called "Nasturtium" of gardens]. We choose for investigation flowers only just opened, because the colour-bodies begin to be disorganized in older flowers. Let us first take surface sections from the upper side of the sepals. The preparation can also be taken with a fine pair of forceps, if these are stuck pretty deeply into the tissue, and a strip torn therefrom. The preparation is laid in a drop of water, with the epidermis turned upwards. Proceed at once to the investigation, because the injurious action of water on the colour-body makes itself felt immediately. The margin of the section will have suffered from the beginning; therefore, cells that are still unchanged should be selected for more searching examination. The colour-bodies are yellow with a shade of orange. They appear spindle-like, three or four angled (Fig. 18), in forms which border on the crystalline. The unchanged bodies are homogeneous. Under the influence of water they swell, become rounded off, and vacuolate; that is, hollows filled with water appear in their interior. The bodies overlie in especial number the inner wall of the epidermal cells of the upper side of the calyx. The brown streaks on the upper side of the sepals proceed, as suitable sections show, from epidermal lines, the cells of which are filled with carmine-red cell-sap. These cells contain also yellow grains, which, however, the coloured cell-sap renders quite invisible. In the red cells the nucleus shows mostly as a clear spot. The petals show analogous relations; here the edges of the limb, as well as the cilia at the base of it, can be used for observation

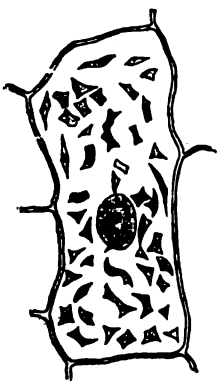

Fig. 18.—From the upper side of the calyx of *Tropæolum majus*. The inner wall of an epidermal cell with the colour-bodies (chromatophores) adjacent to it (× 540).

in their entire thickness. The air adhering to the limb hinders observation, but spots free from air will always be found, or can be made free by light pressure on the limb. The sepals, however always remain preferable for the observation of colour-bodies, since the papillæ interrupt observation of the petals. It is evident that, with the exception of the brown stripes on the two lower petals, every epidermal cell of the upper and under side of it is prolonged in its centre into a blunt cone, the papillæ already alluded to. These papillæ are more strongly developed on the upper than on the under side. They give to the petals a velvety appearance. The air is entangled very strongly between the papillæ. The fiery-red spots at the base of the petals arise from rosy cell-sap and yellow granules. During the investigation it will have been noticed that the surface of the epidermal cells of the upper side is longitudinally striate. The striations do not turn at the boundaries of the individual cells, and are folds of the cuticle which covers the epidermis. With watery solution of iodine the colour-bodies can be fixed pretty well, and take on at the same time a green coloration; they are very sharply defined. The nucleus is at the same time coloured yellowish-brown, its nucleolus becoming very visible. With Methyl violet or with Gentiana violet the colour-bodies are coloured violet.

The yellow colouring matter is almost always combined with a protoplasmic basis; but isolated cases are present where it is met with dissolved in the cell-sap. Let us fix our attention more closely on such a case in *Verbascum nigrum*. We can examine the petals in water without further preparation; but here also we must remove the adhering air, even if only partially, either by pressure or under the air pump. The epidermal cells of both upper and under side have undulating (sinuous) outlines; the yellow colour of their cell-sap is at once noticeable. The brown spots at the base of the petals arise from a cell-sap coloured from purplish to brown. In the epidermis of the staminal filaments, from which lamellæ can be easily cut with the razor, we see also a yellow sap; but besides this there is in each cell also a cinnabar-red irregular lump of colour-material, and a number of colourless leucoplasts filled with starch-grains.

Similarly it can be at once determined that the yellow-coloured parts of the lower lips of the corolla of *Antirrhinum majus* (the Snapdragon) contain a sulphur-yellow sap in their cells; the parts coloured red have a rosy cell-sap and here and there one, seldom more, carmine-red balls of colour-material.

In the epidermis of the corolla of *Vinca major* or *V. minor* (the Periwinkle) we find a blue cell-sap. The epidermal cells, especially of the upper side, are swollen out into papillæ. The epidermis of either side can be readily torn off with the forceps. The side walls of the epidermal cells show ridges projecting into the cell cavity (Fig. 19), often swollen at their edges, so that they can even spread out into a T-form, and, on account of the stronger refraction of their outer surface and the weaker refraction in the interior, quite give the impression of folds.

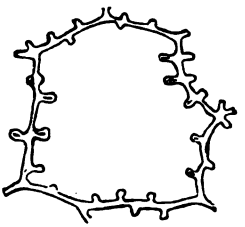

FIG. 19.—An epidermal cell from the under side of the petal of *Vinca minor* (×540).

We see a red cell-sap in the petal of a rose. Here also the epidermis can be readily removed from either side. The upper side has pretty strongly developed papillæ, and therefore appears so beautifully velvety. The cuticle shows strongly marked striation.

In the blue sepals of *Delphinium consolida* (the Larkspur) we find the epidermis of both upper and under sides composed of cells with sinuous outlines. The epidermal cells of the upper side are elevated in their central part each to a papilla. The cuticular striations mount on all sides of this papilla, so that by focussing the microscope at the mid-height of the papillæ, sun-like figures arise. The cells contain a blue cell-sap, somewhat shading into violet, besides also, in many cells, blue stars, which consist of short needles of crystallized colour-substance. The epidermis can be removed in small pieces; moreover, the sepal is sufficiently transparent, after removal of the air, to permit examination at the edges through its entire thickness.

Examples of blue and red cell-sap can be easily multiplied. Such are almost always met with in blue and red flowers; so much the more remarkable, therefore, is the contents of the bright red flowers of *Adonis flammeus*. In *Adonis* also the preparation can be removed with the forceps. In the epidermis we see beautiful red, from nearly round to elliptic, grains; these are comparatively large, and attain the size of chlorophyll-bodies. They appear finely granular, and in water separate quickly into very small granules, which show molecular movements ["Brownian movement"]. The epidermal cells are elongated; their cuticle longitudinally striate; the striæ are clearly continued over the limits of the cells.

The root of *Daucus carota* (the Carrot) furnishes a very interesting object. The orange-red colour of this root arises from carmine and orange-red colour-bodies, which possess throughout a crystalline form. The most common shapes are found collected in Fig. 20. They are small rectangular plates or rhombs, the rhombs often acicularly elongated, and prisms of different lengths, often broadened out at one end to the shape of a fan. Such crystalline formations have often small unilaterally projecting starch-grains attached to them; therefore, these crystalline structures must be placed in the same category with chlorophyll and other colour-bodies. The colour-material which crystallizes out is here, however, what decides the shape. Only a small quantity of protoplasm adheres to the crystal, and from this, therefore, the starch-grains also arise.

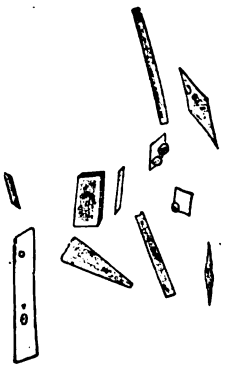

FIG. 20. — Colour-bodies from the root of the Carrot. Partly with starch-grains (×540).

If we examine also one of the variegated forms of our shrubs or trees, or else an herbaceous plant with leaves coloured reddish-brown, we see that the cells of the epidermis contain a rosy cell-sap, and that therefore the joint action of the red of the surface and the green of the interior gives the reddish-brown compound colour.

As to the autumnal coloration of the Virginian creeper, *Ampelopsis hederacea*, we can decide that the rose-coloured cell-sap arises in the cells of the internal tissue, and not of the epidermis. The distinctive yellow autumn coloration of leaves depends on the yellow coloration of the disorganized chlorophyll-bodies, as is shown in the most beautiful way in the leaves of *Ginkgo biloba* [*Salisburia adiantifolia*], or, failing this, those of the various species of Maple. The autumnal brown coloration of leaves arises from a corresponding coloration of the cell-walls, chiefly, however, of the cell-contents, as is easy to determine in the case of the Oak.

The starch-grains are found in specially individualized protoplasmic structures. We have already learned to know the chlorophyll-bodies as such, also the colour-bodies in which starch-grains are often present; and lastly, we have already made reference to the colourless starch-builders. Upon these last devolves the

formation of starch in the deeper layers of the body of the plant. We can comprise all three structures under the name of **Chromatophores**, and, further, distinguish the chlorophyll-bodies, colour-bodies, and colourless starch-builders as **Chloroplasts, Chromoplasts,** and **Leucoplasts** respectively. These structures are nearly related, and can pass over into one another. They all belong to the protoplasm of the cell, and lie embedded therein. On the other hand the blue stars, which we found in the cell-sap of *Delphinium consolida*, do not belong to this; they only represent colour-material crystallized out from the cell-sap, and are, like the lumps of colour-material which we found in the red cell-sap of *Verbascum nigrum*, not to be reckoned amongst the chromatophores.

The largest and most beautiful starch-grains are produced by leucoplasts; but such leucoplasts are not exactly easy to see. A comparatively favourable object, and one not difficult to obtain, is furnished in the rhizome of *Iris germanica*. Surface sections of this are made parallel with the surface of the rhizome. The outermost layer of tissue is removed, and to this succeed the starch layers. The observation is best made in water. In uninjured cells the leucoplasts appear as collections of protoplasm at the hinder end of the starch-grain (Fig. 21). These latter increase only at this end, and have a proportionally eccentric structure. The leucoplasts appear granular to the eye of the observer, and separate at length into smaller grains, which show molecular movement [Brownian movement]. Two starch-grains on one leucoplast is a not infrequent appearance. After further development such grains presently come into mutual contact, and receive thenceforth layers which are common to the two. These and similar phenomena lead, here and in other cases, to the formation of compound starch-grains.

FIG. 21.—Starch-builders with starch grains from the rhizome of *Iris germanica* (×540).

NOTES TO CHAPTER IV.

1. Boehm's method. *Sitzungsber. d. K. A. d. W. in Wien*, Bd. XXII. p. 479.
2. According to A. Meyer, *Das Chlorophyllkorn*, p. 28.
3. A. F. W. Schimper. *Bot. Zeitung*, 1880, col. 881; 1881, col. 185; 1883, col. 105, and 809. A. Meyer, *Das Chlorophyllkorn*, *Bot. Zeitung*, 1883, col. 489.

CHAPTER V.

TISSUES; THICKENING OF THE WALLS; REACTION FOR SUGAR; INULINE, NITRATES, TANNIN, LIGNIN.

MATERIAL WANTED.

White Beetroot (*Beta vulgaris*). Fresh.
A ripening Pear. Fresh.
Tuber of Dahlia (*D. variabilis*). Fresh.
Tuber of Dahlia placed in meth. spirit, in or about October.
Oak-apples or Oak-galls. Fresh and dried.
Twig of Willow (e.g., *Salix caprea*). Fresh.
Stems of Periwinkle (*Vinca major*). Fresh, cut off close above the ground.
Seeds of *Ornithogalum sp.* such as *O. umbellatum*, the Star of Bethlehem.
Seeds (stones) of Date (*Phœnix dactylifera*).
Old Pine wood of any kind, preferably the Scotch Fir (*Pinus sylvestris*). Dry, or, better, in alcohol.

WE commence with the white Beetroot (*Beta vulgaris*). A small piece of tissue is taken from the fleshy root, and from this is made a microscopical preparation. We choose as best for examination a radial longitudinal section, *i.e.*, therefore, a section which is taken parallel to the long axis, in the direction of the radius. This section cuts at right angles the concentric rings of the root, visible to the naked eye. Examined in water, this section shows us more or less rectangular **cells**, filled with a watery, colourless fluid. On the walls of these cells we notice also, here and there, larger and smaller, brighter, round or oval spots, which indicate shallow **pits** *i.e.*, local thin places, or hollows, in the wall. In individual cells the nucleus is visible. The **intercellular spaces** are usually filled with air, appearing black. In isolated parts of the preparation, the parenchymatous cells are narrower, elongated parallel to the long axis of the root; between them are visible long tubes usually filled with air,

which are distinguished by a characteristic thickening of their walls. These tubes are **vessels**. The thickening of their walls is a network of pits [**reticulated**]; that is, the wall shows thickening bands combined into the form of a net, between which lie unthickened places. These unthickened places or pits are elongated across the longitudinal direction of the vessels. Where the section has opened a vessel, there can be seen in it, from time to time, **annular** (ring-like) thickenings, which project into the interior of the cells. These are the diaphragm-like remains of originally complete partition walls, and from these remains it will be seen that the vessel has proceeded from a row of cells. The air present in the vessels often disturbs the examination; it can be got out with the air-pump. When an air-pump is not at our disposal, we can endeavour to remove the air by laying the preparation in freshly boiled water. It is more quickly attained by a short immersion of the preparation in alcohol. It is true that by this the contents of the cells are killed; for the foregoing observation, however, this is not of consequence.

Here and there also in the preparation we come across particular cells, which are closely filled with small clinorhombic **crystals**, and appear almost black. These crystals consist of oxalate of lime. In order to prove this, we allow acetic acid to act upon them, and determine that they are insoluble in it. Into another preparation we run sulphuric acid, and the crystals are quickly dissolved. The quantity of sulphate of lime formed is so small that it remains dissolved in the surrounding fluid.

The structural relations of the cells in the Beetroot show up still more beautifully and distinctly if the section is treated with a watery solution of aniline green or of acetic aniline green. In both cases the cell-walls are beautifully stained green; in the latter case the nucleus also is "fixed" and quickly stained. The walls of the parenchymatous cells, and of the vessels, are alike stained bluish-green. The surface of the pits in the walls of the parenchymatous cells, on the other hand, is not stained, and these therefore now show up more clearly; they are places, in the otherwise not greatly thickened cell-walls, which have remained thin. Each parenchyma-cell contains **nucleus**, provided with a distinct **nucleolus**, and surrounded by minute **leucoplasts**, and a thin lining ["peripheral"] layer of protoplasm. The vessels contain neither nuclei nor plasmic contents. If chlorzinc-iodine is added to a section lying in water, a characteristic violet **cellulose-**

reaction is soon set up. The coloration begins at the edges of the section, but is often not complete for hours. The walls of the vessels do not stain violet, but brownish-yellow; they behave like lignified membranes. On the walls of the cells, the surfaces of the pits once more remain unstained, and stand out specially distinctly. These pit-surfaces are always rounded, of variable size, and irregularly distributed, singly or in groups. Large pit-surfaces are traversed by violet striæ of various breadth; they are formed into compartments by them, and give the impression of an irregular lattice. Bright granules, coloured yellow-brown by the chlorzinc iodine, adhere in larger or smaller quantity to the pit-surfaces. For the purpose of comparison we proceed now to the cellulose reaction with iodine and sulphuric acid. The section is first impregnated with iodine solution, best with potassium-iodide iodine solution, and afterwards transferred to diluted sulphuric acid (English), in the proportions of 2 volumes acid to 1 volume water. It commences at once, from the edges onwards, to indicate the action; the section assumes a beautiful blue colour. The lesser pits here also remain uncoloured; the larger ones appear latticed with blue.

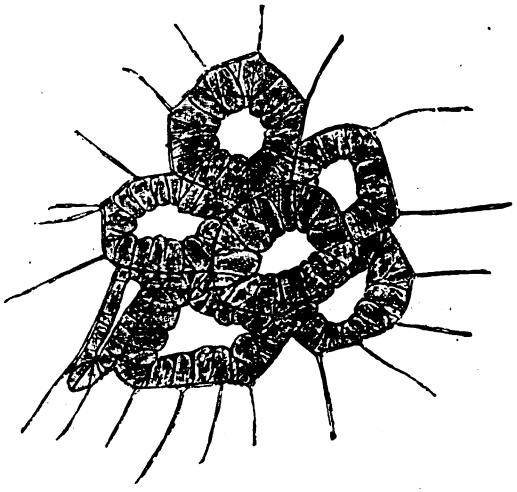

Fig. 22.—From the flesh of the fruit of the Pear. Strongly-thickened cells with branched pore-canals, surrounded by thin-walled parenchyma (× 240).

We further prepare a section from a ripening Pear. Constituting the pulpy flesh of the fruit appears here also a regular thin-walled parenchyma of large cells, more or less rounded at the angles. These cells contain colourless cell-sap, a very reduced plasma-sac, and a nucleus. Scattered in the tissue are found nests of strongly-thickened cells (Fig. 22). The number of the stone cells so united is varied from part to part, and according to the kind of

pear. They form the so-called "grit" of the pear. The cells are distinguished by the considerable thickness of their walls, and by the numerous, fine, branched **pore-canals** [**canaliculi**]. The branching arises from the diminution of the number of the pore-canals proportionally as the cavity of the cell becomes smaller by the great increase in thickness of the walls, so that they open into the cell-cavity as common canals. Where two thickened cells are in contact, it can be determined that the pore-canals correspond in position with one another. In their perfected condition, in which they here appear to us, these cells no longer contain living cell-contents, but only a watery fluid. They represent, therefore, only dead cell-cases. After treatment with chlorzinc iodine, the thin parenchyma-cells take on gradually a violet coloration, the strongly thickened cells become yellow-brown. These latter are therefore lignified and belong, on account of their strong thickening and lignification, to the **sclerenchyma** or **mechanical tissue.** The structural relations of the thickened cells become especially clear under treatment with chlorzinc iodine.

We will use the flesh of the pear in order to learn to know the micro-chemical reactions for **sugar.**[1] That most commonly used is with Fehling's solution. This is prepared with sulphate of copper and potassio-sodic tartrate in water. The proportions are 34·64 gram. pure sulphate of copper with 200 gram. potassio-sodic tartrate dissolved in water. This solution can be preserved. In order to use it we add 600 ccm. soda ley of specific gravity 1·12, and dilute it to 1,000 ccm. This solution is heated to boiling. The section in which the reaction is to be produced should not be too thin, should contain at least two layers of uninjured cells, and naturally should not have previously been laid in water. Immerse the section, holding it with the forceps, in the boiling solution, and the section is coloured a beautiful vermilion-red. The reaction comes out in full beauty after two seconds. Under the microscope we can see in the cells the vermilion-red precipitate of reduced protoxide of copper. There is therefore present in the cells of the pear a substance which reduces the alkaline copper-oxide solution, a body from the grape-sugar group (**Glucose**), in this special case **grape-sugar.**

For comparison we repeat the experiment with a section of Beetroot. This contains, as is known, a body from the cane-sugar group, viz., cane-sugar. Immersed for two seconds in the boiling fluid, it shows no precipitate in the cells; the section, examined

microscopically, has a blue coloration. If the section is kept for a longer time in the Fehling's solution, it begins to colour vermilion-red on the surfaces also. The cane-sugar is inverted, and now gives the protoxide precipitate. Under the microscope the outer layers of cells show now vermilion-red grains, while, in case the action has not been too long continued, the inner cells still contain a blue fluid.

Very much recommended also for microscopical purposes is Barfoed's sugar reaction [2] with acidulated acetate of copper. This solution is prepared by dissolving 1 part of neutral crystallized acetate of copper in 15 parts of water. To 200 ccm. of this solution is added 5 ccm. of an acetic acid which contains 38 per cent. of glacial acetic acid. In a test-tube which holds from 5 to 8 ccm. of this solution we allow a section, not too thin, of the Pear, and in another similar test-tube a section of the Beetroot, to boil for a short time. The fluid in question, together with its section, is then poured out into a small evaporating dish, and allowed to stand. After some hours we find the section of the Pear covered with a fine precipitate of protoxide of copper, and likewise a little of the same precipitate in the evaporating dish, while the section of the Beetroot, as can readily be seen under the microscope, is free from the adhering precipitate, and this is wanting also in the evaporating dish. The result of the reaction should be observed after some hours, as after a longer time a very small precipitate re-oxidizes in the air, and can then dissolve.

We will lastly again use the Beetroot, in order to learn to know the micro-chemical reactions for **nitrates** and **nitrites** by means of diphenylamine.[3] This reagent, used by the chemist for the detection of very small quantities of nitrates and nitrites, performs, moreover, first-rate service for histological purposes. We prepare cross or longitudinal sections through the Beetroot, taking care, however, that the sections extend to the surface. These sections we allow with advantage to previously become somewhat dry on the object-slide, and then first add the reagent. We use 0·05 gram diphenylamine in 10 ccm. pure sulphuric acid. Immediately after the addition of this a deep blue coloration, formation of aniline-blue, shows in the outermost zone of the section. This zone contains the youngest tissue of the root, still in course of development; it is this, therefore, which contains the nitrate. From the parts coloured blue the colour quickly flows over the rest of the preparation, but in the first moment of the reaction the coloured

zone is quite sharply delimited. As, however, in plants, as analyses of sap show, the question is commonly of a nitrate, seldom of a nitrite, we can, therefore, from the resulting reaction, conclude with greater probability that it is a nitrate. If, instead of the somewhat dried section, a fresh one is used for the reaction, the colour-body which is formed is diffused far more rapidly in the surrounding tissue, and the coloured zone is less sharply delimited.

As the next object of investigation we choose the tubers of the Dahlia (*D. variabilis*). The tuber, halved longitudinally, allows one readily to recognise the **central pith**. A longitudinal section prepared from this shows under the microscope more or less rectangular cells, arranged in longitudinal rows (Fig. 23), with very reduced protoplasmic sac, with nucleus, and colourless cell-sap. The intercellular spaces are filled with air; the cell-walls finely striate. The striæ are oblique, to the extent of from 35° to 40°. We believe that we can see two diagonally opposed systems of striæ in the same plane; this is explained by the comparatively small thickness of the walls. In fact, the two opposing systems of striæ belong to the walls of two contiguous cells respectively, as can be determined especially at the free edges of the section. With chlorzinc iodine the cell walls soon colour violet; where, however, two striæ come less closely together, a colourless line can be seen between them. The parts of the wall which remain unthickened are, just like pit-surfaces, not coloured by the chlorzinc iodine solution. Especially clearly show up individual comparatively larger rhombic places as pits. Such pits lie always at the crossing places of two broader lines of separation. A cross-section of the same tissue shows also on the end walls of the cells, which we then see in surface view, no appearance of striation. It shows only here and there larger rounded pits.[a]

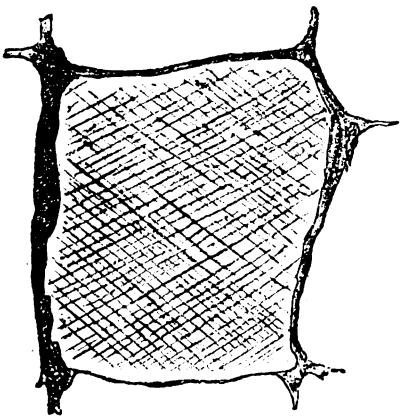

FIG. 23.—From the pith of *Dahlia variabilis* (× 240).

If the section is laid in absolute alcohol there arises in the cell-sap a fine precipitate of **Inuline**. Replace the alcohol by water,

[a] See note on page 60.

and warm the object-slide over a spirit flame, and the precipitate is again dissolved. In order to study the inuline in the shape of **sphæro-crystals,** which it forms,[a] we examine best pieces of tubers which have been placed in spirit at least eight days before. We examine the section best in water, and during the examination allow nitric acid very slowly to enter. The sphæro-crystals (Fig. 24) are found always on the cell-walls. They form more or less perfect balls. The ball can be traversed by one or by several cell-walls. Usually several variously sized balls form together a larger group. The balls allow more or less clearly a radial structure to be recognised; this structure comes out more sharply when the nitric acid begins to work; it arises from radially arranged needle-shaped (**acicular**) **crystals,** which compose the ball. Besides the radial, a concentric stratification is also usually visible, which is to be conceived as the expression of variations in the conditions of crystallization. Iodine solution produces no coloration. If the sphæro-crystals are warmed in a drop of water on the object-slide they quickly vanish.[b]

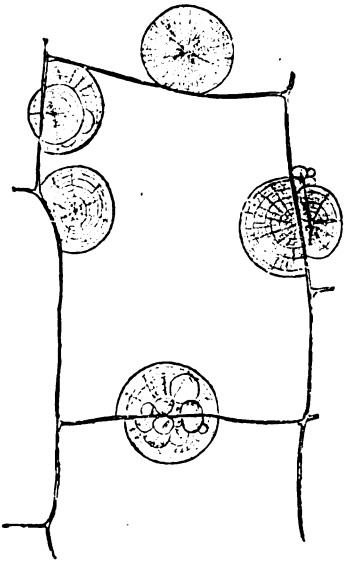

FIG. 24.—From the tuber of *Dahlia variabilis*, after lying in spirits for many months. Sphæro-crystals on the walls (× 240).

In order to demonstrate the **tannin**-reaction upon a typical object, we turn to the **gall-apple** or oak-gall, as it is to be found upon the leaves of our oaks. These gall-apples are due to the puncture of the oak-gall insect [*Cynips querci*], which lays an egg in the punctured tissue. We halve such a gall-apple while still young, and find on delicate radial sections taken from this that the interior hollow, occupied by the larva of the *cynips*, is surrounded by a shell, which consists of iso-diametric, rounded cells. These contain usually abundant starch-grains, becoming blue with iodine. The tissue following on to this inner portion is formed of radially elongated, polygonal cells, which diminish in length at the periphery of the gall-apple, and finally end under the small-

[b] See note on page 60.

celled outermost layer, the epidermis, the cells of which are strongly thickened outwardly. This entire tissue, surrounding the inner shell, shows no enclosures of definite form. If, however, we lay a freshly-prepared section in a drop of watery chloride or sulphate of iron solution, we see that it colours throughout its entire mass of a dark-blue colour. This coloration is, moreover, communicated to the surrounding fluid, and produces for us, therefore, the iron reaction for tannin, in its **iron-blue** form, while there is also an **iron-green** form. If the action is observed under the microscope, by allowing iron-solution to run into a dry section laid under a cover-glass, we see that first a fine dark-blue precipitate is formed, which, however, is soon again dissolved in the reagent, so that now a blue fluid fills the cells. The weakest tannin reaction is given by the starch-containing cells of the inner shell. For comparison, let us now lay a second section in a watery solution, about 10%, of bichromate of potash, and we see a dense flocculent, red-brown precipitate, which also persists, formed in the tannin-containing cells. Lastly, let us place a section in a concentrated solution of molybdate of ammonia, in concentrated ammonium chloride, and an abundant reddish-brown precipitate appears in the cells. This reaction will decide in doubtful cases, because those preceding can also proceed from other reducing bodies. The **fibro-vasal bundles** which traverse the oak-apple we will leave unnoticed, and also pass over other structural relations, because we have only taken this object in order to see a typical tannin reaction. Sections of dried gall-apples also give the above reactions, though less beautifully.[c]

In order to get the iron-green tannin reaction, we take a willow twig, say from *Salix caprea*, remove with the razor the outer green layer of bark, and then take a delicate tangential section from the green tissue of the cortex; lay it in a drop of chloride of iron solution. The section shows us mostly rectangular cells, somewhat elongated in cross direction, with walls pretty strongly thickened, and with simple pits. These cells contain chlorophyll-grains, and most of them, especially in winter, have each a white strongly refractive rounded mass of cell contents, sharply defined, and filling the entire cell cavity. Other isolated cells contain a dark-looking stellate crystal of calcium oxalate, of which we shall, however, have an opportunity later of making a closer examination. The strongly refractive masses of cell-contents contain tannin. As soon as the action of the iron chloride on the strongly refractive masses of

[c] See note on page 60*a*.

cell-contents has commenced, these become grumous, and take on an olive-green to brown-green colour. In iron sulphate these masses become still browner; in potassium bichromate they give a reddish-brown precipitate; in ammonium molybdate dissolved in strong ammon. chloride, a yellow-brown grumous precipitate. With twigs of the alder (*Alnus*) the same results are produced.

If a strong stem of *Vinca major* [the Periwinkle], cut off close above the ground, is broken, we see from the edge of the broken surface numerous small fibres project. We seize a number of such fibres with the forceps, draw them out, and place them in a drop of water on an object-slide. Under the microscope they appear to us as long, strongly-thickened **sclerenchyma-fibres**, tapering at both ends. The cavity is reduced to a narrow canal, which is entirely obliterated at both ends of the fibre. In slightly-thickened fibres the wall appears striate in one direction only. In more strongly-thickened fibres there are two oppositely oblique systems of striæ, of which one belongs to the outer, the other to the inner system of wall-layers [complex of lamellæ]. Lastly, in still older sclerenchyma-fibres is often found still a third internal system of striæ, directed almost perpendicularly to the long axis. This last arises from reticulated thickening bands, which leave between them elongated pits. This innermost system of thickening is usually sharply limited towards the exterior ones. With chlorzinc iodine solution the fibres take on immediately a violet coloration, passing into brown. Specially instructive, however, is the relation with cuproxide ammonia, which reagent has the power of dissolving pure cellulose. The action must be observed directly. On the addition of the cuproxide ammonia solution the walls of the fibres swell strongly. At the first moment of the action the striation becomes more distinct, but quickly disappears. The outer complexes of layers are soon completely dissolved, while the inner reticulated one resists longer, and therefore the observer sees it completely isolated. At the beginning of the swelling a still finer stratification appears in the stratification which was previously visible. Each **layer** is therefore composed of numerous exceedingly thin **lamellæ**. Such a fine stratification is stamped especially distinctly upon the inner more resistent layer.

We now divide in halves, with the pocket-knife, the seed of *Ornithogalum*, say *O. umbellatum* [the Star of Bethlehem], clamp the half in the hand-vice, damp the cut surface with water, and make with the razor the thinnest possible preparation. This

preparation (Fig. 25) presents us cells with approximately rectangular contour. The walls of these cells are strongly thickened, the thickening layer being, however, pierced by numerous simple pits. If the section has so grazed a cell-wall that it presents a surface view, the pits appear as round pores (*m*), as can be seen in the upper cell of the adjoining figure. From the side the pits appear as canals, which pass out of the cell-cavity up to the primary cell-wall. The pits of adjoining cells are directed towards one another; they are separated by the primary wall (*p*), which we shall here designate the **closing membrane**. The inner surface of the thickening layer is distinguished by stronger refractiveness; it forms the **limiting membrane**. If sulphuric acid is allowed to act slowly on the preparation, from the edge of the cover-glass, the thickening layers of the cells are dissolved, while a network of very delicate walls is at first left behind. The walls are the so-called **middle lamellæ**, which indicate the walls of the cells which were present before the thickening began, and which also traverse the closing membrane of the pits. By continuous action of the sulphuric acid, these middle lamellæ also soon disappear. Chlorzinc iodine causes the thickening layers to swell and the middle lamellæ become likewise visible. In consequence of the swelling, the coloration of the preparation is incomplete.

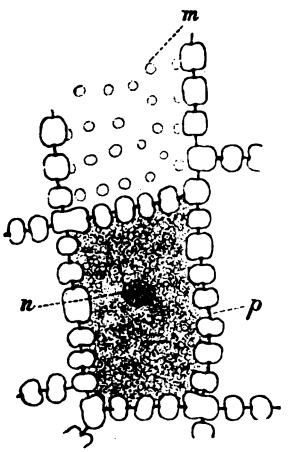

FIG. 25.—From the endosperm of *Ornithogalum umbellatum*. *m*, pits seen from above; *p*, closing membrane in pits seen in profile; *n*, nucleus (× 240).

The cells are closely filled with protoplasm and granular materials. These entire contents take on with iodine a greenish-brown coloration. In each cell the nucleus is readily distinguishable with acetic aniline green; this is, in general, wanting in no cell, living or capable of life.

The thickening layers of the cells in the endosperm of the Date (*Phœnix dactylifera*) have a very similar appearance. The cells, however, are more elongated, their cavity narrower, the walls somewhat thicker. In the seed ["stone"] of the Date these cells are radially arranged. Cross and longitudinal sections of it,

therefore, provided they correspond with the radii, show the cells in longitudinal view, while tangential sections, which cut the radii at right angles, show cross-sections of the cells. Chlorzinc iodine solution colours the thickening layers very beautifully violet. By slower swelling usually numerous lamellæ are brought into sight.

We turn now to the pine wood [*Pinus*, etc., any species, preferably *P. sylvestris*, the Scotch fir], in order to learn to know **bordered pits**. For this purpose we take a piece of wood, either dry, or, better still, preserved in alcohol, from a stem as old as possible. First we prepare with a sharp pocket-knife the suitable surfaces for cutting—one radial, parallel to the long axis of the stem, one tangential to the same, and one directed perpendicularly to this axis. The concentric yearly rings which are visible with the naked eye upon every piece of pine wood will provide us with the necessary bases from which to get information as to the directions in question. The radial longitudinal section cuts the yearly rings perpendicularly. The tangential longitudinal section is so much the more perfect, the more parallel it runs to the yearly rings. The cross-section is directed perpendicularly to both longitudinal sections. In the following preparation of microscopical sections, in order that the sections shall be good, and not to damage the razor, quite special precautionary rules must be adopted. If the razor is hollow-ground, rightly directed sections can be taken only from the edges of the piece of wood, *i.e.*, so long as the back of the razor does not yet rest upon the cut surface. However, in general, only slightly hollowed razors should be used for cutting wood, as those greatly hollowed easily "give." It is recommended to use razors which are ground flat on one side, *i.e.*, the side which will rest upon the cut surface; but these razors have the disadvantage that they are not easily sharpened. The cut surface must always be moistened; the sections must be as thin as possible. It is not necessary to have them of any particular size. A section which appears to be too thick should not be cut to the end; it is better to withdraw the razor from the cut in order not to notch the edge. The razor must be sharp, otherwise it will tear the cell-walls, and separate the inner thickening layers from the outer. The wood preserved in alcohol cuts more easily than when dry, especially when the former has been laid subsequently in a mixture of equal parts glycerine and alcohol. The surface of the cut surface prepared by the pocket-knife, as it contains the torn cell-

walls, must be removed with the razor. The succeeding sections can be used.*

A radial longitudinal section, correctly taken through the wood of the Pine, appears, with weak magnification, to be constructed of longitudinally elongated cells, which overlap one another with their tapering ends. Running across these cells we see the cell-rows of the **medullary rays**, with which we shall not at present concern ourselves. We focus now with stronger magnification upon a part in which we see only the walls of the longitudinally elongated **wood-cells** [**fibres**], and always the broader of them, and direct our whole attention to the **bordered pits** of these walls. The bordered pit appears to us in the form of two concentric circles (Fig. 26, *A*). The inner small circle, or, it may be, the inner ellipse, indicates the opening of the pit into the cavity of the cell; the larger outer circle, or outer ellipse, the widest part of the pit, with which it joins on to the primary wall separating the two cells. In fact, this pit is only distinguished from the simple pit, as we have seen it in the Date and in *Ornithogalum*, in that it broadens at its base. The pits of the adjoining cells, however, meet here in just the same fashion. If the mouth of the pit, as commonly, is an obliquely placed ellipse (as in *A*), by changing the focussing we shall find the corresponding mouth of the other pit oblique in the opposite direction. The two **pit-chambers** adjoining one another are separated from one another by the primary wall, which, before the commencement of the secondary thickening, was already pre-

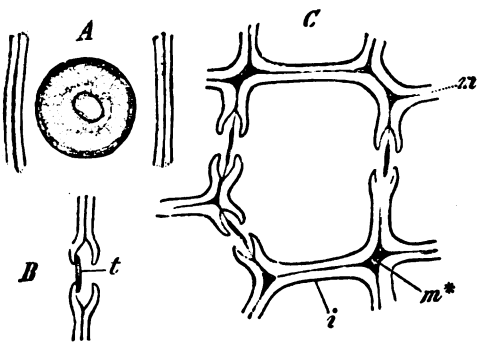

FIG. 26.—*Pinus sylvestris*. *A*, a bordered pit in surface view. *B*, a bordered pit in tangential longitudinal section; *t*, the torus. *C*, cross-section of an entire tracheïde; *m*, middle-lamella; *m**, a "seam"; *i*, the limiting membrane (× 540).

* It is of some advantage to keep an old razor (sharp, however,) for preparing surfaces, as it is keener than a pocket-knife, and will spare the actual section razor. Even then the first section cut with the latter should be rejected. [ED.]

sent, and subsequently is only slightly thickened. This delicate wall is the **closing membrane**. In the middle it is more strongly thickened, and forms the so-called **torus**. With most careful observation and suitable focussing we may even be able to see this torus. It forms a round, weakly-shining disk, which has about twice the diameter of the mouth (compare in *A*). In the most favourable cases, and here especially in preparations of dried wood, a radial striation is observable in this torus, and so that the delicate part of the closing membrane appears differentiated into radially dispersing lamellæ.[6]

A complete insight into the structure of the bordered pit can only be obtained with the aid of tangential sections. As the bordered pits stand on the radial walls of the wood-cells,[7] they are seen in cross-section (Fig. 26, *B*) in correctly taken tangential longitudinal sections. We search for these structures in the walls separating the wood-cells, stopping first at the dividing walls of the broader wood-cells, and not allowing ourselves to be led astray by the sectional view of the medullary rays, which are formed of a number of smaller cells, standing one over the other. The figure of the cut pit is, it is true, clear only in very delicate parts of the section. If this condition is fulfilled, the pit appears in the form of the two ends of a pair of tongs directed towards one another [or like a couple of extremely short screws placed with their heads flat together], after the type of the above figure (26, *C*). If once the structure of this large bordered pit is known, we can obtain information as to the structure of the smaller ones, which lie in the thicker walls of the narrower wood-cells. The difference, apart from the smaller size, is, that here on both sides a longer canal, corresponding to the thickness of the wall, runs out of the broadened pit-chamber. The largest bordered pits are connected with the smallest by all intermediate stages. In the interior of the pit is seen, in the most favourable cases, the closing membrane, which in its centre is swollen into a torus (*t*). In the bordered pits of the air-dry wood it is usually pressed to one side of the pit-chamber (*B*). If, on the other hand, fresh wood, or alcohol material, is investigated, we shall find the closing membrane in the sap-wood [alburnum] stretched across the middle of the pit-chamber. In the heart-wood [duramen], on the contrary, the relations are just as we have given for the air-dry wood. The figure of the bordered pit is clearer after the action of chlorzinc iodine, which stains the cell-wall yellow-brown. This coloration

is due to the strong lignification of the walls. Only in occasional places is a violet tinge still to be seen there, *i.e.*, where a not yet completely lignified inner thickening layer gives this colour reaction. The closing membrane is in general not stained by the chlorzinc iodine. After treatment with this reagent we can readily convince ourselves that the perfect wood-cells contain here neither protoplasmic sac nor nucleus; they consist only of dead cell-walls, and, as they functionally contain only water, and in this respect, as well also as in the nature of the thickening of their walls, they simulate the tracheæ, *i.e.*, vessels, they are known as **tracheïdes**, more recently as **hydroïdes**.

Not infrequently the pine wood, which we examine, shows in longitudinal section a more or less distinct spiral striation mounting at an angle of about 45°. The mouths of the pits then appear elongated in the direction of the striation, and as do the striæ of the two adjoining side-walls, so also the mouths themselves of adjoining pits cross one another.

We prepare a cross-section also of the pine wood. This must be specially thin. The tracheïdes thus cut across appear as a rule rectangular. They form radial rows. We pause at one with the widest lumen (cavity). On its radial walls we see the sections of the pits (Fig. 26, *C*), the figure of which appears in no way different from the tangential longitudinal section. Between the cells the **middle lamellæ** proceed as fine separating lines (*m*). Where more than two cells are in contact, the middle lamella is thickened into a solid or hollow "seam" (*m**). The inner limit of the cell-wall is more strongly refractive and forms the **limiting membrane** (*i*), which is specially clear in the more strongly thickened tracheïdes with narrower cavities. It is always clearer after the action of concentrated sulphuric acid. The thickening sheaths swell, and are finally dissolved; the limiting membrane resists longer and stands out sharply. Between the swelling thickening layers are seen the primary walls of the cells, of which finally only the delicate network of middle lamellæ is left behind, stained yellowish-brown. These middle lamellæ, resisting concentrated sulphuric acid, are **cutinized** [**cuticularized**]. With slower swelling in sulphuric acid it can be often determined, and especially on the strongly-thickened tracheïdes, that the thickening layer consists of very numerous extremely delicate **lamellæ**. With chlorzinc iodine the cross-section, as previously the longitudinal section, is coloured yellow-brown; in individual cells, how-

ever, part of the thickening layer impinging directly upon the limiting membrane, takes on a violet tone. If we follow the treatment with chlorzinc iodine with dilute sulphuric acid (two parts acid, one part water), under the influence of this latter a blue coloration of the entire thickening layer is possible. If delicate sections are treated with concentrated chromic acid, an opposite action to that of sulphuric acid results. The middle lamellæ are dissolved, and the individual cells, therefore, are separated from one another. The thickening layer of the cells undergoes a not inconsiderable swelling; the limiting membrane at the commencement of the action stands out sharply, but soon becomes unrecognisable. [d]

In order further to learn the characteristic reactions for **Lignin**, we will make use of phloroglucin and of sulphate of aniline.[8] We dissolve a trace of phloroglucin in alcohol, and lay some sections of wood in this solution. After this we place it in a drop of water on the object-slide, and allow, from under the edge of the cover-glass, hydrochloric acid to act upon it. The walls of the cells quickly take on a beautiful violet-red coloration. Other sections we place in a watery solution of aniline sulphate, where they at once become bright yellow; this colour is still more heightened by the addition of dilute sulphuric acid. In place of the phloroglucin we can use an extract, prepared with water or spirits of wine, from the wood of the Cherry, with almost the same result.[9] If fresh sections of the stem of the Pine, passing from cortex to pith, are treated with concentrated hydrochloric acid, a yellow coloration of the wood is at once brought about, which, however, gradually shades off, inwardly and outwardly respectively, into a violet coloration.[10] This also is the phloroglucin reaction, and indeed proceeds from the phloroglucin which comes from the contents of the cortical cells and pith cells respectively. Even the medullary rays of the young wood contain a little phloroglucin, so that the violet coloration also spreads from each of these.

In the future, we shall make use of the different relations of lignified and unlignified cell-walls towards certain colour-bodies as an assistance in our investigations.

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[d] See note on page 82a.

NOTES TO CHAPTER V.

[1] Compare Sachs, most recently in *Jahrb. für wiss. Bot.* Bd. III. p. 187.

[2] *Barfoed de organiske Stoffers qualitative analyse.* Kjöbenhavn. 1878, pp. 210, 217, 223. Notes.

[3] Compare H. Molisch, *Ber. der deutsch. botan. Gesellsch.* I. Jahrg. p. 150.

[4] Sachs, *Bot. Ztg.* 1864, p. 77; Hansen, *Arb. d. Bot. Inst. in Würzburg.* Bd. III. p. 108; Meyer, *Bot. Ztg.* 1883, Col. 334; W. Gardiner, *Proceedings of the Cambridge Philosophical Society.* Vol. IV., Pt. VI. p. 387.

[5] Sanio, *Jahrb. f. wiss. Bot.* Bd. IX. p. 50. Strasburger, *Zellhäute*, p. 38. Russow, *Bot. Centralbl.*, 1883. Bd. XIII., Nos. 1–5. The other literature is there quoted.

[6] Compare Russow, *Bot. Centralbl.*, 1883. Bd. XIII., Nos. 1–5.

[7] Bordered pits placed on the tangential walls occur rarely in the Pine, but on the contrary are quite regularly met with in the autumn wood of the other Abietineæ.

[8] Both introduced by Wiesner (compare *Stzbr. der math. nat. Klas. der Akad. der Wiss. zu Wien.* Bd. LXXVII. 1. Abth., and before that in other places).

[9] Von Höhnel, *Stzber. der math. nat. Kl. der Wiener Akad. d. Wiss.* Bd. LXXVI. p. 685.

[10] The same, page 676.

[Note to page 50.]

[a] It is a very widely-spread phenomenon that the cross-walls in a tissue are thickened differently to the longitudinal walls, a phenomenon especially corresponding with the differing demands which are made upon their permeability for the conduction of food materials, etc.

[Note to page 51.]

[b] The sphæro-crystals of dahlia tubers are not always of inuline. In tubers preserved in alcohol, bulky sphæro-crystals of calcium phosphate are often deposited. They have the same form, but usually smaller size than those of inuline, and are slowly dissolved in water if the preparation be examined therein, while their refractive power continually decreases. The sphæro-crystals of calcium phosphate are either without clear lamination and distinct nucleus, or they show an amorphous nucleus, which is surrounded by a shell of acicular crystals. In the latter form the nucleus of the sphæro-crystal is deeply stained by carmine solution; in the former the sphæro-crystal is stained throughout its entire mass, and in this manner can again be made visible after it has almost disappeared through the action of water. In the one case the organic substance is confined to the nucleus; in the other it is pretty uniformly distributed through the entire mass of the sphæro-crystal. These sphæro-crystals rapidly disappear when acted upon by nitric acid. Concentrated sulphuric acid turns them brown at once, and then quickly changes them into an array of crystals of gypsum, while the sphæro-crystals of inuline, on the other hand, appear unchanged.

If the dahlia tubers do not show sphæro-crystals of calcium phosphate, they can be readily found in other plants. They are certain to be found in the ground-tissue of the fleshy Euphorbias, cultivated in plant-houses, such as *E. Caput-Medusæ*, when this has been laid in spirit. They are found very beautifully in the pith of the inflorescence of *E. helioscopia* (the sun-spurge), when this is preserved in spirit.

[*Note to page* 52.]

[c] More roundabout, but specially certain, is the reaction with acetate of copper. The material to be investigated is, while living, cut up into small pieces, and placed in a saturated solution (7 p. c.) of acetate of copper, and allowed to lie therein for eight or ten days, or longer. Sections then taken are laid upon a slide in a drop of 0·5 p. c. solution of sulphate of iron. In this they remain only for a few minutes, as, after longer action, the walls commence to go brown. After the sections have been washed in water, and placed in a watch-glass of alcohol, in order to remove the air and chlorophyll, they are examined in glycerine. The sections remain unchanged in glycerine and glycerine-jelly. The pieces of plant can be removed from the acetate of copper into alcohol, and examined later at pleasure by the aid of acetate of iron. Iron-blue and iron-green tannins are clearly distinguishable by this method.

[*Note to page* 59.]

[d] With slow swelling in sulphuric acid it can be determined, especially in the strongly thickened autumnal tracheïdes, that the thickening layer consists of very numerous extremely thin lamellæ. In chlorzinc iodine the cross-section, like the longitudinal section, is coloured yellowish-brown, the middle lamellæ pure yellow. In isolated cells the part of the thickening layer immediately bounding the limiting membrane, may take on a violet tone. If the action of chlorzinc iodine is followed by that of dilute sulphuric acid (two parts acid to one part water), a blue coloration of the whole thickening layer is often induced.

Specially delicate cross-sections of pine-wood furnish excellent "test-objects," with the aid of which we can form an estimate of the quality of the medium and high powers of our microscope. Assuming that we have a sufficiently thin section, the figure must appear thoroughly flat, well illuminated, sharp in outline, clear in the details of its structure, and free from colour.

CHAPTER VI.

THE EPIDERMIS, STOMATA*, WATER STOMATA.

MATERIAL WANTED.

Leaves of *Iris florentina*. Fresh.
Leaves of *Tradescantia virginica*. Fresh.
Leaves of *Aloë* (e.g. *A. nigricans*) or *Agave*. Fresh.
Leaves of *Aeonium* (e.g. *A. fraxinifolia*). Fresh.
Leaves of *Nerium oleander*. Fresh.
Leaves of *Tropæolum majus* (Indian cress, or so-called "Nasturtium", fresh, or in alcohol.

TAKE a surface section of the outer side (morphologically the under side) of the "equitant" leaves of *Iris florentina*. The section must be so thin that it only grazes the tissue underlying the epidermis, and should be observed in water with the outer side turned upwards. It will be at once seen that the **Epidermis** is composed of elongated cells which run parallel to the long axis of the leaf. The cells are ended by cross partition walls; they are connected together without any intercellular spaces (other than the stomata), contain colourless cell-sap, a nucleus, and a very reduced protoplasmic sac. On its external side the epidermis is covered by an exceedingly fine-grained layer of wax. In a line with the cells of the epidermis lie the elliptic **Stomata**, which, however, are only indistinctly visible because the four cells of the epidermis which surround each spread over the **Guard-cells** of the stoma, partially covering them. Hence there remains only an elliptically elongated pit (*f*) which leads to the stoma (Fig. 27, *A*). This pit usually appears black, because filled with air. In order to see the guard-cells well, now turn the section over. It can then be easily proved that the stoma is formed by two half-moon-shaped guard-cells. In distinction from the neighbouring epidermal cells these cells contain chlorophyll bodies. The nuclei are wont to show as clear spots about the mid-length of the cells. Between the two guard-cells is a spindle-shaped cleft (*s*), about half the length of these cells. Since the long axis of the stomata corresponds with the long axis of the leaf, it is easy to obtain correct

* In ordinary terminology, the term "stoma" includes the guard-cells and the accessory structures. I have limited the term, as etymologically it should be limited, to the cleft ("stomatic cleft," of many authors). The whole structure I have described as "stomatic apparatus." What I call here the "air-chamber," underlying the stoma, is the "respiratory cavity" of many authors. [ED.]

cross sections of the stomata. The section is taken at right angles to the long axis of the leaf. For this purpose a narrow strip, about $\frac{1}{8}$-inch broad, should be cut out of the leaf in the direction of its length with a pair of scissors; this strip can be supported between two pieces of the pith of the elder or of the sunflower.* The elder or sunflower pith necessary for this purpose is obtained from dried pieces of the stems of those plants by stripping off the cortex and woody bundles. A piece of pith about an inch long is cut in two lengthwise with a sharp razor. The flat strip of tissue

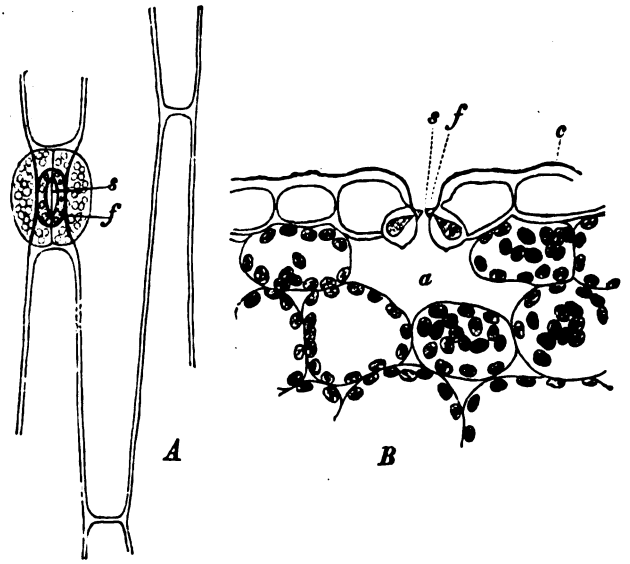


FIG. 27.—Epidermis of the under side of the leaf of *Iris florentina*. *A*, surface view; *B*, in cross-section. *f*, stomatic pit; *s*, cleft, or stoma; *c*, cuticle; *a*, air-chamber (× 240).

which has to be cut is now laid between the two halves of the pith, so that the end of the strip reaches to the end surface of the piece of pith. Thin cross sections are then taken through pith and object at the same time, and the sections are lifted with a camel hair brush from the blade of the razor on to the object-slide. While cutting, the two pieces of pith can either be held together simply with the fingers, or the two halves can be fastened together by tying round with a piece of thread. In cutting, the pith is so

* Or, several such strips can be packed together without other support than they give to each other. [ED.]

held that the razor lights on the broad side and not on the thin end (edge) of the object; in this way many equal sections can be taken. For delicate objects the softer sunflower pith is preferable to the somewhat harder elder pith; for more resistant objects, like that in question, elder pith is better used; for still more resistant objects, not pith, but fine cork, as used for bottles.* The preparation of sufficiently thin sections need in this instance present no real difficulty; under any circumstances such difficulty can be overcome by the use of a *Microtome*. A hand microtome of the simplest construction, such as Zeiss (of Jena) offers in his Catalogue for 1883, as No. 140, at 18*s*., would suffice. This has a round cutting plate, ground smooth, of about three inches in diameter, which is fastened to a cylindrical tube, likewise serving as a handle. Inside this tube is placed a second, movable upwards and downwards by means of a screw. The extent of the movement can be read off upon a divided disk. The pieces of pith, between which the object is, are placed between two pieces of cork, hollowed out to receive them, and these are firmly fixed in the inner tube of the microtome. The pieces of pith project somewhat beyond the pieces of cork, and reach as high as the upper cutting plate. The sections can be taken either with an ordinary razor, or with one ground flat on one side; and while one hand holds the microtome, the razor is moved with the free hand over the cutting plate. In the case of the razor with one side ground flat, this side should be applied to the cutting plate. Sections are best cut by pushing the razor away from the operator.

* A few more practical hints on the subject of section-cutting by hand may be of use to beginners. The razor for most purposes should be what is called "hollow-ground," and of tolerably good quality, *and should be kept sharp*. The object to be cut should be held pretty firmly between the thumb and index-finger of the left hand, the index-finger being held as nearly as possible horizontally, and slightly bent, the thumb likewise very slightly bent, and with the joint depressed below the level of the finger, in order to secure its safety should the razor slip. In holding the object to be cut, the side of the tip of the index finger should be rather higher than that of the tip of the thumb. The razor being then grasped firmly but not stiffly, the blade held quite flat and horizontal, the edge towards the body; the index-finger of the left hand will serve as a table, on which the blade will lie and thus be greatly steadied. The section should be cut by a single forward and lateral movement of the blade. With all objects which will bear it, the razor-blade may float with alcohol on its upper side, and the object should be similarly wetted; otherwise the object, as here, may be kept moist with water. For this purpose two "wash-bottles" are a saving of time—one for distilled water, the other for alcohol. [Ed.]

After cutting each section, the object should be somewhat raised by turning the screw. Microtomes of complex construction, such as are necessary to zoologists, are superfluous for botanists.

In this way a considerable number of sections are prepared for further use, and they can be laid in the meantime, by means of a camel-hair pencil, in a watch-glass filled with water. Place some of the sections in water for observation under the microscope, and they will show, in favourable places, median cuts through the stomata, as shown in Fig. 27, *B*. As such a cross section will show, the epidermal cells of *Iris florentina* are more strongly thickened on their outer than on their inner side. The inner walls, however, are also pretty thick, while the radial walls are only slightly thickened. This structure is connected with the function of the Epidermis, which not only has to serve as an outer protecting sheath, but also has to functionate as a water-reservoir.[2] The thin radial walls easily allow a change in the capacity of the cells, which, by means of a bellows-like play, diminish in height through loss of water, and enlarge again with increase of water. The guard-cells lie recessed between the epidermal cells; the manner in which the latter overlap the guard-cells can be at once seen. The pit leads down to the guard-cells. These latter show a cross-section quite peculiar to them. On the upper and under side they are strongly thickened. These thickened places are contiguous on that side on which is the stomatic cleft. Above each of these places is found a peculiar beak-like projection. On the opposite side, turned towards the interior of the epidermal cells, the guard-cells are comparatively thin-walled. This method of thickening of the wall is connected with the mechanism of the movement of the guard-cells, which would more strongly curve, and thus widen the cleft, when their turgidity increases, but which would straighten themselves, and thus diminish the cleft, when their turgidity decreases. It is clear, indeed, that with increasing turgidity the guard-cells must become more convex on the side of less resistance, more concave on the side of greater resistance; just as an indiarubber ball, with a wall thicker on one side, must, by the forcing in of water or air under high pressure, become concave on the side of stronger resistance. The thin place on the side of the cleft, where the two thickened parts join together, facilitates the flattening of the cells on this side during curvature. In order that the movement of the guard-cells may not be prejudiced, we see the outer epidermal

wall join on to these guard-cells with suddenly diminishing rim; the guard-cells are here fastened as with hinges,—the **epidermal** or **stomatic joints**, or articulations.[a] Under the stoma is found the air-chamber (*a*), a large intercellular space, under natural conditions filled with air, surrounded by chlorophyll-containing cells, and connected with the intercellular spaces which are found between them. A cross-section laid in chlorzinc iodine shows us that the walls of the epidermal cells stain in their entire extent, with the exception of a thin outer layer, somewhat corrugated, the so-called **Cuticle** (*c*) which becomes yellowish-brown. This cuticle swells out at the stoma into the beak-like projection which we have already mentioned, which appears coloured yellow-brown by the chlorzinc iodine, and is therefore cuticularized. As an extremely delicate membrane, the cuticle is continued through the stomatic cleft, over the guard-cells, to the commencement of the chlorophyll-containing parenchyma. For the rest, the guard-cells are also violet over their whole extent, and are therefore of cellulose. By the use of concentrated sulphuric acid the whole section is dissolved, the cuticle alone remaining behind, together with the cuticularized projections of the stoma.[b]

An exceedingly favourable object for the study of the stomatic apparatus is found in *Tradescantia virginica*. The epidermis on both sides of the leaf consists of polygonal cells, mostly elongated in the direction of the long axis of the leaf; with these alternate narrow stripes of longer and narrower cells. These stripes are visible with the naked eye, especially on the under surface of the leaf, and appear green in colour, while the stripes of broader cells show grey. The lateral walls of the epidermal cells are pitted; the outer surface is faintly striate. The number of stomata is markedly greater on the under side of the leaf; therefore we choose this side for investigation. The stomata are always surrounded by four epidermal cells (Fig. 28, *A*). The guard-cells lie on the same level with the epidermal cells; the cleft which they have between them is comparatively large; they contain chlorophyll-grains, between which the nucleus is usually visible. In the epidermal cells also the nuclei are sharply conspicuous, and appear surrounded by colourless leucoplasts (Fig. 28, *A*, *l*); the cell-sap of the epidermal cells is here and there rose-coloured. The long axis of the stomata corresponds with the long axis of the leaf, so that here also it is easy to obtain correct cross-sections. The stomata present then the appearance shown in Fig. 28, *B*.

[a] [b] See notes on page 71.

The stomatic side of the guard-cells here also appears to be thickened, while the side turned towards the interior of the epidermal cells is thinner. Besides this, it happens that both of the epidermal cells bounding the guard-cells are flatter than the epidermal cells lying beyond, and are also less thickened on their outer sides. They appertain, therefore, to the stomatic apparatus as "**accessory cells**"; they form the hinge or joint which in *Iris florentina* is formed merely by the thin part of the membrane at the insertion of the guard-cells. The leucoplasts (*l*), which surround the nucleus in the epidermal cells, offer here a very favourable object for observation. It is interesting that these leucoplasts, in spite of being in a position so strongly exposed to the light, remain small and colourless, and do not develop into chlorophyll-grains. The epidermis has here another purpose, and has not to functionate as an apparatus for assimilation.

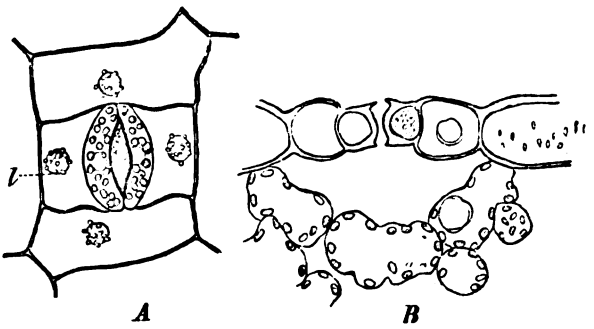


FIG. 29.—Epidermis of the under side of the leaf of *Tradescantia virginica*. *A*, seen from above; *B*, in cross-section through the leaf; l, leucoplasts upon the nucleus (× 240).

The so commonly cultivated *Tradescantia zebrina* has a stomatic apparatus composed in the same way. Stomata are present only on the under side of the leaf. The cross-section is very instructive, though not easy to obtain thin; thicker sections serve for general information. The epidermal cells on both sides of the leaf are alike distinguished, as cross-sections show, by their considerable size. Those on the upper side especially are so deep that they alone form half the thickness of the leaf. Many of these epidermal cells are seen to be divided by cross-walls. On both sides of the leaf the epidermal cells contain little besides watery cell-sap, that on the under side, moreover, mostly appears coloured red. The

leaves of *Tradescantia* present, therefore, in their epidermis, a specially efficacious water-reservoir. The accessory cells of the stomata, almost always four in number, are, as the cross-section shows, quite thin, so that a great air-chamber, of the depth of the surrounding epidermal cells, is formed under the stomatic apparatus. In thicker parts also of a surface section, taken from the under side of the leaf, the form of the air-chamber can be traced out by deeper focussing, so long as the chamber is not opened by the razor, and remains filled with air. The leucoplasts around the nucleus of the epidermal cells are again clearly visible.[c]

The species of *Aloë* and *Agave* possess epidermal cells thickened very strongly on their outer sides, and stomata correspondingly deeply sunk in the epidermis. Because it is specially instructive, and not difficult to prepare, we select for observation *Aloë nigricans*, a greenhouse plant with ligulate leaves arranged in two series (ranks). Other species of *Aloë* can, if need be, serve as substitutes for this. In surface sections, the epidermis of upper as well as under side appears formed of regular polygonal cells, mostly hexagonal. The cavity (or lumen) of each of these cells is reduced to a relatively small, rounded space. This space appears dark, because the razor opened the cells from below, and the cavities filled with air. The stomata are found on both sides of the leaf; deep pits lead up to them. These pits are always bounded by four cells, and have a rectangular contour; a somewhat projecting rim surrounds the pit. If you wish to see the guard-cells, it suffices to lay the section on the glass slide with the inner side upwards. The guard-cells are comparatively broad and short; amongst their contents are noticeable strongly refractive spherical oil-globules. As the epidermis is very hard, the cross-section is best taken between two pieces of bottle cork. The whole thickness of the leaf need not be taken, but rather a piece of the tissue, about $\frac{1}{25}$th inch thick, is cut off from one surface of the leaf. As the stomata run parallel to the long axis of the leaf, we arrange the piece of leaf so that it shall be cut at right angles to this axis. We cut the sections from the inner towards the outer, *i.e.*, from the soft towards the harder part of the tissue. The strong thickening of the epidermal cells is observable immediately in these sections (*see* Fig. 29); this thickening affects only the outer half of the cell; corresponding to it, the cavity of the cell tapers in an outward direction. The thickened parts of the cell-wall are white, strongly refractive, and are covered externally by a cuticle more

[c] See note on page 71.

strongly refractive still, but not sharply delimited. The lateral boundaries of the cells are only indicated by delicate lines in the thickened mass, and outwardly by a slight ridge. The interior of the strongly refractive thickening sheath is clothed by a comparatively slight, weakly refractive layer (*i*). This surrounds, therefore, first the keel-shaped lessening part of the cell-cavity; while gradually thinning off, it ends in the side walls simultaneously with the refractive thickening layer. This thickened part of the epidermis, viewed in the aggregate in the section, appears like a curtain cut into regular teeth. At the places where the hollows leading up to the stomata are found, is first to be noticed the projection which encloses the hollow as with a rim; next, that the tooth, formed by the thickening layers, is here halved unilaterally, and has also only half its usual depth. The guard-cells show, both above and below, on the stomatic side, projecting ridges, which in cross-section appear beak-like. Above the guard-cells are found the thin parts of the wall which serve as epidermal joints. The air-chamber is narrow and deep. Commonly a parallel, more or less oblique, striation will be observed on the thickened walls of the epidermal cells; it is caused by the razor in cutting, and recurs in the same way not infrequently on hard elastic objects. A section treated with chlorzinc iodine, shows the highly-refractive thickening layer coloured yellow-brown; it is, therefore, cuticularized. The inner covering to this layer (*i*) is, on the other hand, coloured violet, as likewise is the rest of the tissue of the leaf. The yellow-brown coloration passes over the "hinge" on to the projections which are on the guard-cells above and below. Elsewhere the guard-cells are coloured violet. On treatment with concentrated sulphuric acid, the whole of the part which colours yellow-brown with chlorzinc

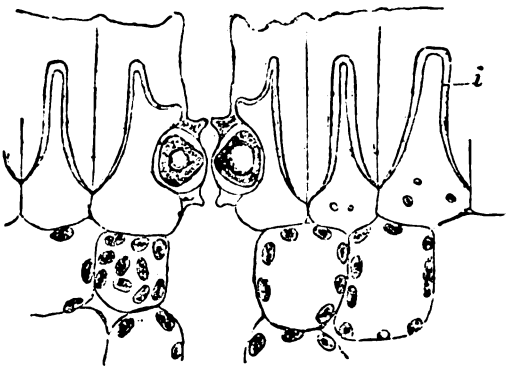


FIG. 29.—Cross-section through the epidermis and stoma of *Aloë nigricans*. *i*, inner thickening layer (× 240).

iodine remains at first behind; after some hours' action this also is dissolved, and then the delicate cuticle, and the fine middle lamellæ found between the epidermal cells alone still persist. The cuticle is continued over the guard-cells to the junction with the chlorophyll-containing inner cells. The cuticular layers and the cuticle take a brown colour in the sulphuric acid. The oil present in the guard-cells "balls" together, immediately on the entrance of the acid, into a highly refractive spherule, which disappears after some time.

Many modifications occur in the arrangement of the stomata in the epidermis. A very remarkable instance is that where the stomatic apparatus is surrounded by a single annular epidermal cell. This can be observed in *Aneimia fraxinifolia*, a fern which is to be found in every botanical garden. The cells of the epidermis have a strongly undulating ["sinuous"] outline (Fig. 30), and, by this mutual dovetailing, so common a thing in epidermal cells, gain in firmness and solidity. Like all other ferns, *Aneimia* contains chlorophyll-grains richly in its epidermal cells. Here, therefore, such a division of labour as exists in most Phanerogams is not carried out, and the epidermis forms part of the assimilating tissues. The stoma is set in the surrounding epidermal cell as in a frame. Cross sections (at right angles to the lateral veins) show us that they project somewhat above the surface of the epidermis. This extreme case is connected by intermediate forms, with other less remarkable ones, into which we shall not further enter. We need only to imagine the stomatic apparatus removed to the side wall of the surrounding epidermal cell, to do away with the unusual character of their insertion.

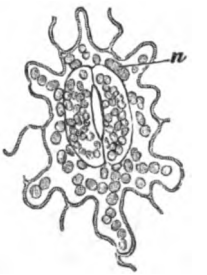


FIG. 30. — *Aneimia fraxinifolia*. Stoma, surrounded by an epidermal cell; *n*, nucleus of the epidermal cell (× 240).

Nerium oleander shows a peculiar condition. Neither on the upper nor the under surface of the leaf can stomata at first be seen. On both sides we find a comparatively small-celled epidermis, which, especially on the under side, is covered with unicellular hairs, their walls thickened almost to the disappearance of the cavity. On the under side of the leaf, however, there appear also larger or smaller depressions, filled with air, and garnished at their edges with short hairs, resembling those just mentioned, but with less thickened walls. These hairs, coming together,

close up the aperture towards the exterior. A second surface section from the under surface of the leaf, taken from the same place, whence a previous one has already removed the epidermis, permits to us here and there a view of the bottom of the hollows. For this purpose it is, above all, necessary that the air should be previously removed from the hollows, either under the air-pump, or through soaking the section in alcohol. It is then shown that from the walls of the depression, project small conical elevations, whose apex is formed by a stoma. The side walls of the small cones consist of epidermal cells, which allow between them an air-chamber extending to the stoma. Between the cones bearing the stomata, the similar hairs to those which we have seen on the edges spring from the walls of the cavities.

We will now turn our attention to a specially favourable object for observing **Water-Pores** or **Water-Stomata.** These show the same structure as the air-stomata, but are larger, the cleft, as well as the adjoining intercellular space (air-chamber) is, at least partially, filled with water. The guard-cells of these stomata may be from the first immovable, quickly perish, and then at all events lose their movability. The most favourable object for the study of these water-pores is *Tropæolum majus* [the Indian cress or so-called "Nasturtium"]. The water-stomata are found in the upper side of the leaf, and always over the ends of the principal veins (or ribs). Here the edge of the leaf usually shows a small depression. A pretty clear view of the water-stomata can be had if a suitable piece of a leaf throughout its whole thickness is brought into the field of the microscope, under water, and covered over with a cover-glass. The details are indeed only observable on surface sections taken from the proper part of the edge of the leaf. A water-stoma then presents the appearance

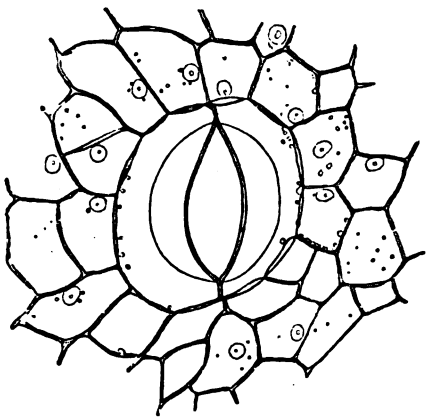


FIG. 31.—Water-stoma of the edge of the leaf of *Tropæolum majus*, together with the surrounding epidermal cells (× 240).

in Fig. 31. The contents of the guard-cells were in this case already reduced to a minimum. Several water-stomata are always found at a short distance from one another.

NOTES TO CHAPTER VI.

[1] Strasburger, *Jahrb. für wiss. Bot.* V. p. 297; de Bary, *Vergl. Anat.* pp. 32 et seq.; 70 et seq. (See trans. by Bower & Scott, pp. 29, et seq.; 66 et seq.) Schwendener, *Monatsber. d. kgl. Akad. d. Wiss. in Berlin*, 1881, p. 833. For the remaining literature, see the two first-named authorities.

[2] Westermaier, *Jahrb. für wiss. Bot.* XIV. p. 13.

[*Notes to page* 65.]

[a] Nevertheless it lies also under the control of the turgidity existing in the neighbouring epidermal cells, which has a preponderating influence in determining the width of the cleft for the time being.

[b] In the same way the cuticle resists strong chromic acid, in which, however, it soon becomes very transparent. Cold potash cannot dissolve cuticle, which resists it even better than cork does. For the rest, "cuticularized" and "converted into cork" are quite equivalent ideas.

[*Note to page* 67.]

[c] Very beautiful large stomatic apparatus are present on the under side, more rarely the upper side, of the leaves of the white garden lily, *Lilium candidum*, and this can therefore be recommended as an object for investigation. The epidermal cells are elongated in the long axis of the leaf, lie in straight rows, but have, however, an undulating outline. The stomatic apparatus stand in the prolongations of the epidermal cells, and at the same height with them. The cross-section is easy to obtain, and shows a hinge at the point of junction of the guard-cells in the form of a sudden thinning of the strongly thickened outer wall of the neighbouring epidermal cells.

CHAPTER VII.

THE EPIDERMIS (CONT.); HAIRS.[a] MUCILAGE AND WAX.

Material Wanted.

Young branches of Wallflower (*Cheiranthus Cheiri*). Fresh.
Leaves of Ten-week Stock (*Matthiola annua*). Fresh.
Flowers of Pansy (*Viola tricolor*). Fresh.
Flowers of Mullein (*Verbascum nigrum*). Fresh.
Leaves of *Verbascum thapsiforme*. Fresh.
Leaves of *Shepherdia canadensis*, or of *Eleagnus angustifolius*. Fresh.
Young stems of *Rosa semperflorens*, or other rose. Fresh.
Young stems of the stinging nettle (*Urtica dioica*). Fresh.
Leaf-stalks of the Primula, *P. sinensis*. Fresh.
Young stems of *Rumex patientia*. Fresh.
Leaves of Sundew (*Drosera rotundifolia*). Fresh.
Winter buds of *Æsculus Hippocastanum*.
Leaves of *Echeveria secunda-glauca*, or other like kind. Fresh.
Piece of cortex of node of sugar-cane (*Saccharum officinarum*). Fresh.

We are already acquainted with the **root-hairs** of *Hydrocharis morsus-ranæ*, and as with root-hairs it is always a case of similar unicellular sacs, we can abstain from further investigation of them. We have also seen the epidermal cells of numerous petals elongated into conical **papillæ** (*Tropæolum*, Rosa), and also the **staminal hairs** of *Tradescantia*, threads formed of barrel-shaped, swollen cells (Fig. 15); lastly also the hairs of *Cucurbita*, passing over from a multicellular base into a simple pointed thread. Plant hairs are therefore known to us from many points of view; it is, however, worth while to extend our special knowledge of them.

On the leaves and stems of **Cruciferæ** we find very many forms of much-branched **unicellular hairs**. On the stems and leaves of the Wall-flower, or Gilly-flower (*Cheiranthus Cheiri*), we see spindle-formed structures (Fig. 32, *A*), with narrow cavities obliterated towards the two ends. These unicellular spindles are covered on their outer surface with protuberances, always fewer large ones

[a] See note on page 82.

with numerous small ones between. As the spindles are all directed parallel to the long axis of the leaf, it is comparatively easy to obtain a good cross-section through them. It is indeed desirable to hit upon the hair at its point of insertion in the centre of its length, and numerous sections must therefore be taken in order to increase the chance of success. Then we see (Fig. 32, *B*) that the place of insertion of the hair lies somewhat depressed, and that the epidermal cell which broadens out outwardly into the body of the hair is smaller than its neighbours, that at the base it is somewhat swollen, rounded, and reaches more deeply into the surrounding tissue. It forms the "foot" of the hair. Longitudinal sections through the leaf show that the foot is not broader in the long direction of the hair than in the cross direction. We can readily satisfy ourselves that the cavity of the foot passes without interruption into the cavity of the body of the hair. We can obtain a still more complete figure of the form of the foot if we lay a thin surface-section with the under side upwards. The foot is circular in cross-section. It can now be seen, also, that the chlorophyll-containing cells of the tissue of the leaf adjoin radially, and without interruption, the somewhat broadened part of the foot projecting below the epidermis.

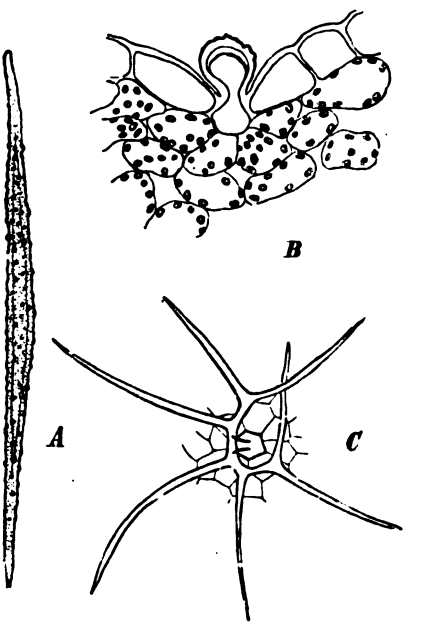


FIG. 32, *A* and *B*.—From the under side of the leaf of *Cheiranthus Cheiri*. *A*, the hair seen from above (× 90). *B*, in cross-section (× 240). *C*, from the under side of the leaf of *Matthiola annua*; hair seen from above (× 90).

The hairs of the ten-week stock, *Matthiola annua* (Fig. 32, *C*), are repeatedly branched in one plane. These hairs, especially on the under surface of the leaf, are set so closely together that their branches interlace. The cavity of the hair, in consequence of the strong thickening of the walls, is well-nigh obliterated. Knobs

are scarcely at all developed on the surface. The view of the epidermis from the inner side (by means of surface sections placed upside down) is very instructive, for it shows a tolerably marked swelling of the globular foot of the hair, and around it an exceedingly beautiful radial arrangement of the chlorophyll-containing cells.

In the groove of the lower spur-like elongated petals of the pansy (*Viola tricolor*) are very peculiar long unicellular hairs (Fig. 33). They can be seen very well if a cross-section of the lower petal is taken near the place where the tubular spur opens out into the furrow or groove. Each of the epidermal cells concerned grows out, almost in its entire width, into a hair. This is covered with irregular knotted swellings. The membrane of the hair shows slight longitudinal ridges. The cell-sap is colourless, but yellow pigment-bodies (chromatophores) are often present in the protoplasmic sac.

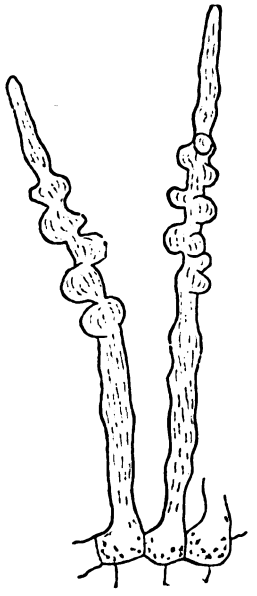


FIG. 33.—Hair from the furrow of the lower petal of *Viola tricolor* (× 240).

The staminal filaments in the flowers of the common Mullein (*Verbascum nigrum*) are covered with unicellular violet hairs. In order to examine them the anther should be removed from the filament, and this latter pulled to pieces with needles in a drop of water on an object-slide. The hairs are very long, swollen out at the end into the form of a club, and with violet **cell-sap.** The surface of the hair is covered with elongated protuberances which ascend in more or less regular spirals.

Branched **multicellular hairs** are to be found in the same plant on the under side and edges of the corolla. Seen from above, these hairs have a certain likeness to those of *Matthiola*, but all of the branches here arise from a common central point, and each branch is in itself a closed cell. Moreover, the branches do not spread out in the same plane, but arise at indefinite angles. Their walls are quite as strongly thickened as in Matthiola; outer protuberances are wanting. The hairs on the edges are seen in side view. The body of the hair is cut off by a partition wall from

the epidermal cell which bears it. It consists of a stalk or pedicel, almost always unicellular, and upon this the branches are mounted. Slight modifications of these conditions occur, which need no further explanation. Besides these branched hairs, the edge of the corolla also bears small **glandular hairs**. These have a two to three celled stalk, and a flattened head, which is covered here and there at the apex by a strongly refractive substance. These last we shall not, however, study here, but in another more favourable object.

It is only necessary to imagine the multicellular branched hairs of the mullein placed one upon another several times in order to understand the hairs which form the **felt** on the leaves of *Verbascum thapsiforme*. These hairs are sometimes as many as five stages high, each stage is separated from its predecessor by a unicellular joint, which continues the main axis of the hair. The cells of the hair are for the most part filled with air. They are best shown by cross-sections through the midrib of the leaf. *Verbascum Thapsus*, a native perennial, has very similar hairs.

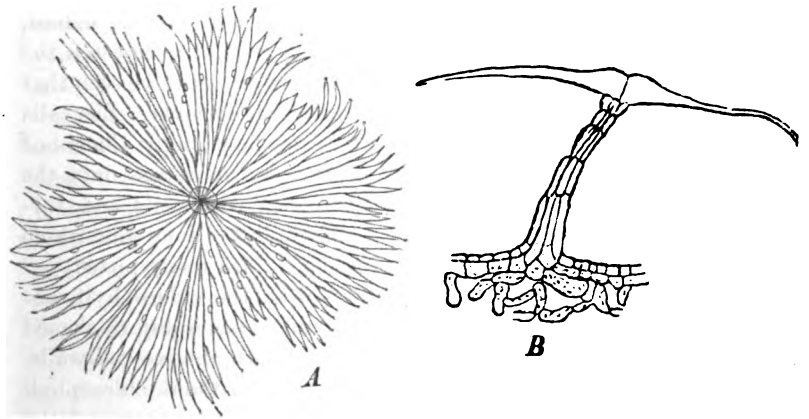


FIG. 34.—Scales from the under side of the leaf of *Shepherdia canadensis*. *A*, from the surface; *B*, in cross-section (× 240).

To the same category as the branched hairs of the petals of *Verbascum* belong the **scales** of *Shepherdia canadensis*. On the under side of the leaf, even distinguishable with a hand-lens, we find more or less loosely-formed white, and more or less closely-formed brown (Fig. 34, *A*) stars. On the upper side of the leaf only white stars are to be found, and they always in small number. The cells of these looser white stars contain, as microscopical examination

shows, only air; they arise from a common central point, but are separated from one another laterally. On the upper side of the leaf they do not lie in one plane, but rather radiate stellately in all directions. The cells of the brown stars are connected together almost to their ends, and provided with living contents; the nuclei in their interior can be seen without difficulty. A cross-section through the leaf, where it cuts a brown star centrally, shows that its stalk (Fig. 34, *B*) is multicellular, and that not only the epidermis but also the cell-layer next following passes over into it. The stalk bears aloft the stellate unilamellar but multicellular expansion.

Should *Shepherdia canadensis* not be at our disposal, *Elæagnus angustifolius* can to a certain extent replace it. Here, on the under side of the leaf, only the white air-containing scales are present. The disk consists of cells either laterally isolated or also grown together almost to the margin.

Now take a horizontal section through the stem of a rose, say *Rosa semperflorens* of the gardens, at the place where one of the prickles arises. Try to halve the prickle as nearly as possible in the middle, and then to take a thin section. This last is, indeed, not so easy as it seems. In cutting, do not neglect to moisten the cut surface with water. In a successful section it can be seen that the epidermis of the stem is continued over the prickle. The cells of the epidermis are at the same time more strongly thickened and more elongated. Inside the epidermis there pass into the prickle pretty strongly thickened narrow-cavitied cells, and, further in, similar but broader ones. These last fill up the whole central part of the prickle. All these cells are finely pitted.[b] The epidermis of the stem is separated from the chlorophyll-containing inner tissue by a strong layer of considerably thickened elongated cells joining on to one another with oblique end-walls, and containing no chlorophyll. These cells without chlorophyll are of like origin to those which form the inner tissue of the prickle. The elements of the tissue of the prickle, are, however, separated from the chlorophyll-containing tissue of the stem by a layer of flat-celled tissue. This strip of tissue arises by division from the undermost layer of the tissue of the prickle; it follows only for a short space the chlorophyll-containing tissue of the stem, and then turns towards the epidermis, in order to bound the base of the prickle laterally also towards the chlorophyll-less tissue of the stem. This is a **cork-layer**, next to the outer surface

[b] See note on page 82*a*.

of which, by the interposition of a layer of separation (**abscisslayer**) the fall of the prickles will result in the older parts of the stem. Before this, it is possible to break off the prickle pretty smoothly from the stem, along the inner side of the cork-layer.

If we select a prickle from the leaf-stalk for investigation, its structure is found to be in no way different from that on the stem, excepting that at its base the cork-layer is wanting. Since the leaf as a whole will fall, separate provision for the fall of the prickles is unnecessary.

By careful examination of the cortical tissue adjoining the prickles of the rose, the presence of **crystals** in the cells can be made out. As they are not dissolved in acetic acid, nor in potash, but on the other hand are dissolved in hydrochloric acid without evolution of gas, they are crystals of oxalate of lime. They have here the form either of monoclinic prisms or of **cluster-crystals.** These last consist of a great number of crystals which are deposited on an original crystal. The cluster-crystals are specially distinguished by their size and stellate form.

In order to get the **stinging hairs** of the common stinging nettle (*Urtica dioica*) uninjured, we must take them from the younger parts of the plant. They are found best on the veins, or ribs, of young actively growing leaves. The hair, which is visible with the naked eye, should be cut off below its point of insertion with the razor, and examined in water. If the hair is already dead, air will be found in its interior, and its apex is then no longer intact. An uninjured hair presents the appearance represented in Fig. 35. The hair is unicellular, sharply conical, swelling at its apex into a small knob. At the base the hair broadens out, and the bulb thus formed is sunk in a cup which is developed from the tissue of the leaf. As its developmental history shows, this hair springs from a single epidermal cell, lying at the same level with its neighbours; afterwards the strongly-swelling foot of the hair is lifted up on a column of tissue, which is covered by the epidermis, and is formed internally of hypodermal (sub-epidermal) tissue. In the hair itself is to be seen **streaming** of the protoplasm. The nucleus is usually to be seen inside the bulb, suspended by protoplasmic threads. The cuticle shows oblique striation, which ascends in the same direction in all the hairs. The wall of the hairs is siliceous, as can be readily proved by heating it red-hot on a mica plate. As already noted, hairs are often found with their points broken off. In case of

careless contact, the hair, by means of this point, enters the skin, and as it is very brittle, breaks off, whereon the strongly acid sap enters the wound and causes slight inflammation. On the same piece of the epidermis, near to the stinging hairs, are also small unicellular bristles (cf. Fig. 35); these last are distinguished by the strong thickening of their walls, and their fine tapering points.

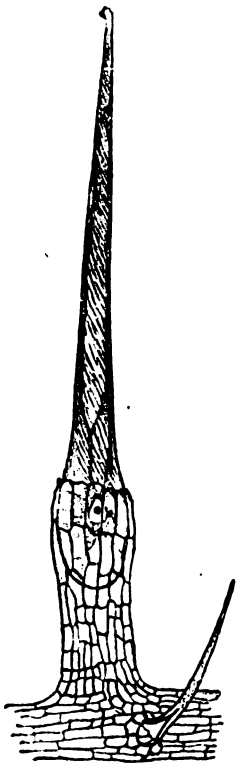


Fig. 35.—Stinging hair of *Urtica dioica*, together with a fragment of the epidermis, on which is a small bristle (×60).

We can find the same kind of bristle on the edge of the leaf. For this purpose it suffices to place a piece of the leaf in water under a cover-glass. In old leaves the bristles can be thickened almost to the obliteration of their cavity; their surface is covered with small protuberances.

We have already met with **glandular hairs** on the edge of the petals of *Verbascum nigrum;* they can be studied under more favourable conditions in *Primula sinensis*. For this purpose cross-sections are taken through a leaf-stalk. The body of the hair is divided from the epidermoid foot-cell by a cross wall situated out beyond the epidermis, and forms a cell row, which consists of usually two (sometimes more), longer, and at the same time broader, and one (rarely two), narrower and also shorter cells. This last cell bears the globular **head**. Upon this, however, is formed a more or less strongly-developed cap of highly refractive resinous yellowish substance. The secretion takes place between the cuticle and the wall of the cell. The cuticle is raised, distended, and finally ruptured, whereupon the secretion overflows the upper part of the hair. The addition of alcohol removes the secretion, and then the raised cuticle can be clearly seen lying in folds. The cells of the hair show a beautiful network of protoplasm with suspended nucleus, in which lies a large nucleolus. Small chlorophyll-bodies are embedded in the peripheral protoplasm. Very beautiful for observation are the glandular hairs (**colleters**) upon the membranous sheathing stipules (**ochreæ**) of

the leaf of *Rumex patientia*, one of the docks not found in Britain. The masses of secretion given off from the glands are here so considerable, that in damp weather the apex of the stem and the young leaves are found entirely covered with slime. The membranous ochreæ can be observed directly, and for that purpose they must be turned with their inner side upwards. A careful examination of the preparation will show the glands in the form of minute plates. These minute plates rise with a short unicellular foot from a small epidermal cell. To the one cell succeed two; upon these usually four cells, which are elongated in the direction of the long axis of the plate, and are repeated in several stages. On the outwardly-turned walls of the cells of the gland are often to be seen bladder-like swellings, which sometimes occupy a part, and sometimes the whole wall of a cell. The secretion, therefore, is formed here also between the cuticle and the rest of the cell-wall, and lifts the cuticle up. At length the bladders open and let out the secretion. This secretion is not coloured by iodine, nor with chlorzinc iodine; in water it swells to a perfectly clear solution, and behaves like a gummy body. The cells of the glands are rich in protoplasmic contents, and their nuclei are distinct. With Rosaniline violet the glands take an intense violet coloration, and the masses of slime are pale-red. Watery solution of nigrosine stains the slime steel-blue, without colouring the glands.[c]

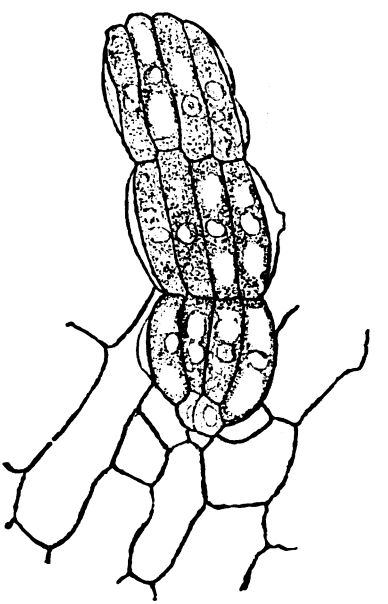


Fig. 36.—Gland from the ochreæ of *Rumex patientia* (×240).

Especially interesting in structure are the glandular hairs of the common sundew (*Drosera rotundifolia*), distinguished alike as **digestive glands** and **tentacles**. They arise as thread-like structures from the edge and entire upper surface of the leaf. The threads (Fig. 37) taper a little in the course of their length, and

[c] See note on page 82a.

swell into the form of an egg at their ends. The threads consist of delicate cells, elongated in the longitudinal direction; the stronger threads are traversed by one or several tubes with screw-like thickenings,—the **spiral vessels.** The radial extension of the epidermis of the thread in forming the head, the fan-like arrangement of the elements of this epidermis, and their multiplication into two or three layers, are seen best in optical sections of the object (Fig. 37). The number of the spirally-thickened vessels is greater in the head; all the cells which lie inside the sheath formed by the division of the epidermal cells, take on this spiral thickening. The place of insertion of the thread, if correctly hit upon, shows that not only the epidermis but also the inner tissue of the leaf is continued into the tentacles. These digestive glands give out a slimy secretion, which clings to the head like a dew-drop

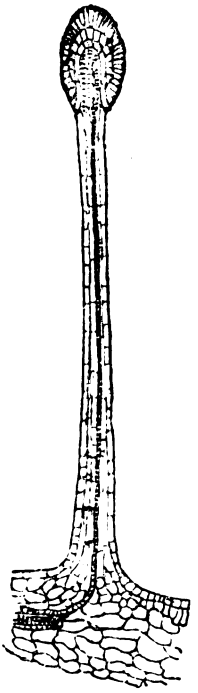
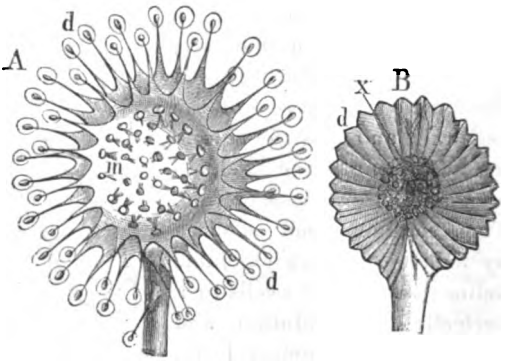


FIG. 37. — Digestive gland [tentacle] of *Drosera rotundifolia* (× 60).



[FIG. 37ª—Leaf of *Drosera rotundifolia*, diagrammatized. *A*, expanded; *d*, tentacles on the edge of the leaf; *m*, the shorter, stouter tentacles of the centre of the leaf. *B*, all the tentacles have bent towards the middle at the touch of an insect, *x* (after Prantl).]

[whence the common name of the plant], but does not arise under the cuticle, but rather flows out from its free surface. Small insects remain sticking in these slime drops, suffocated in the secreted slime, and are carried towards the centre of the leaf by a corresponding inflection of the stalk of the digestive gland. Then the other digestive glands all bend together over the body of the insect, and come in contact with it with their heads. Upon this

the chemical nature of the secretion changes; a free acid and a **ferment,** like pepsin, make their appearance, and these are enabled slowly to digest the albuminous bodies found in the body of the insect. The dissolved substances are absorbed into the plant.

A cross-section through a winter bud of the horse-chestnut (*Æsculus hippocastanum*) shows us button-shaped glandular hairs, situated on the scales covering the bud (Fig. 38). The intermediate scales of the bud bear glands on both sides; on the external ones more are found on the inner side, on the inner scales the most on the outer surface. The structure of the glands is shown in the figure; it shows an axial cell-row, which towards the top divides, and from which the secreting cells radiate. The figure gives the gland in longitudinal section. The cuticle is broken through by the secretion, and this is discharged between the scales, coating them and sticking them together. This secretion consists of a mixture of **gum** and **resin.** In water the gum-drops scattered in the resin can be seen to swell, while on the other hand, by the addition of Rosaline violet, the resin mass is coloured a beautiful blue. Here also the contents of the glands are red.

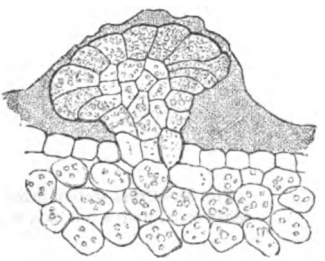


FIG. 38.—Glandular hair on a scale of the winter bud of *Æsculus hippocastanum*, covered with secretion (× 240).

On one object (*Iris florentina*), we have already drawn attention to the finely granular layer of **wax** which covers the outer surface of the epidermis; we propose, however, to investigate this point specially on some other plants.

Very suitable for this is *Echeveria secunda-glauca*, or other like plant, which is now so often used in gardens for "carpet-bedding." The wax layer, which can easily be wiped off, gives to the plant a hoary or "glaucous" appearance. A surface view of the epidermis shows us a net-like crust of aggregated grains.

In an easily observed form, we see aggregated short rods forming a wax layer, in the surface view of the epidermis of *Eucalyptus globulus*, the Australian blue-gum tree.

The most beautiful object is the sugar-cane (*Saccharum officinarum*), now so commonly cultivated in plant-houses. Here the wax covering appears in the form of long rods or filaments, often curved or curled at the end. We remove a surface section

from the nodes of the stem, which are noticeable from their glaucous appearance. As much air clings between the rods, it is best to immerse the section for a short time in cold alcohol. It can be then readily examined. On the other hand, it is difficult to obtain a good cross-section with the rods still adhering. Fig. 39 shows such an one. The rods stand closely crowded against

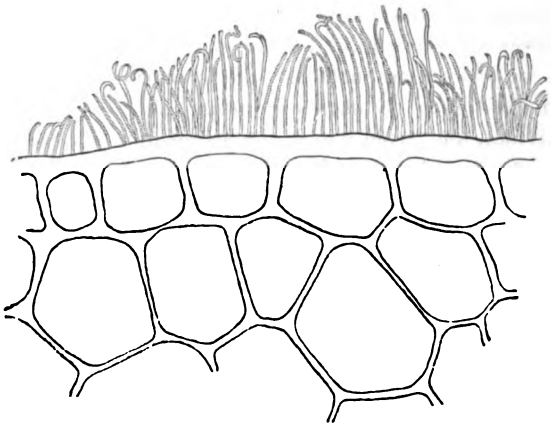


FIG. 39.—Cross-section through a node (knot) of the stem of *Saccharum officinarum*, with a rod-like, waxy layer (× 540).

one another, many showing the bending already referred to. If a surface section is brought into proximity to a flame, the rods, under the microscope, show fused together. They dissolve in hot alcohol.

NOTE TO CHAPTER VII.

[1] Compare this with de Bary, *Comp. Anat.*, §§ 10, 13, 16, et seq., and the literature also given there.

[*Note to page* 72.]

[a] A very favourable object for the study of hairs, and obtainable all the year round, is the petiole of the ordinary zonal bedding geranium, *Pelargonium zonale*. The hairs found upon this, studied in surface and cross-sections, are of several distinct kinds, falling into two groups, viz. conical and capitate. The conical hairs are either (1) delicate and unicellular, (2) stouter, and with one or two partitions, the septum, where solitary, being commonly quite close to the outer line of the epidermis, or (3) very long and tapering, with many cross septa, and a distinctly bulbous base, raised upon a wart-like outgrowth, the cells

of which show radial arrangement. The capitate hairs are (1) glandular hairs with a round head-cell on a short stalk, 2–3 cells long, (2) glandular hairs with an obovate head-cell set somewhat obliquely on a short stalk; (3) doubtfully glandular with a large pear-shaped head-cell on a long 2–3 celled stalk. [ED.]

[*Note to page* 76.]

[b] These cells belong to the sub-epidermal system of the branch. The prickle is an "emergence," involving in its origin sub-epidermal as well as epidermal tissue.

[*Note to page* 79.]

[c] The large stipules of the pansy, *Viola tricolor*, are very deeply toothed, and bear at the apex of each tooth a beautifu egg-shaped gland. If it is desired to see these not shrivelled, but as in active life, it is necessary to investigate the youngest possible stipules. The gland (Fig. 36A) is separated from the edge of the leaf by a somewhat narrowed neck. It consists of two or more rows of

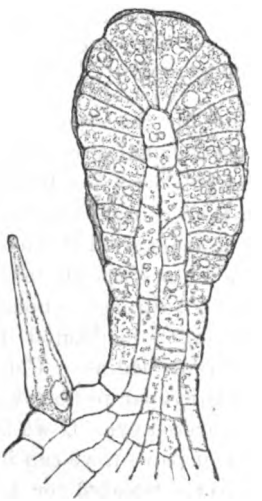


FIG. 36A.—Glandular hair from the stipule of *Viola tricolor*, and an unicellular hair close by (× 240.)

elongated cells, forming a core, upon which a single layer of cells is placed, arranged perpendicularly to the surface, and elongated in this direction. The figure shows such a glandular hair in optical longitudinal section. The whole gland is distinguished by its copious protoplasmic contents. In these are often to be seen vacuoles filled with cell sap, either singly or in groups. The secretion consists of a thin layer of resin on the surface of the gland, and of masses of slime, which raise the cuticle. With rosaniline violet the contents of the cells are coloured red, the resin layer blue, the slime masses reddish.

CHAPTER VIII.

CLOSED COLLATERAL FIBRO-VASAL (OR FIBRO-VASCULAR*) BUNDLES. MUCUS AND GUM.

MATERIAL WANTED.

Stems of the Maize, or Indian Corn (*Zea Mais*), some time in alcohol.
Or, stems of the Oat (*Avena sativa*), or other grass, likewise in alcohol.
Full-grown leaf of *Iris florentina*, some time in alcohol.
Stem of *Dracæna* (*Cordyline*) *rubra*. Fresh.

A VERY favourable object for the study of the structure of the **closed collateral fibro-vasal bundles**[1] of the Monocotyledons is the stem of the Maize or Indian Corn (*Zea Mais*). We will investigate material which has lain for a considerable time in alcohol, in order the more readily to become acquainted at the same time with the cell-contents. First prepare a cross-section, taking care that it passes through an internode and not through a node. The comprehension of the structure will be much facilitated if the section is laid at once into a drop of chlorzinc iodine. Coloration of the section immediately begins, and the separate fibro-vasal bundles stand out quite clearly, even to the naked eye. If we lay the glass slide on a white object [*e.g.* a sheet of paper], we can in the readiest possible way get information as to the **isolated** (or scattered) arrangement of the fibro-vasal bundles; an arrangement, as a whole, peculiar to monocotyledons. It will also show that the fibro-vasal bundles are more closely crowded together towards the periphery of the stem. Every fibro-vasal bundle shows in cross-section as an oval spot: the tissue in which these bundles are embedded is the **Ground**, or **Fundamental Tissue**. A separation of the ground-tissue into pith and cortex is not present with the scattered or isolated arrangement of the bundles. Now find, under the micro-

* In the course of the chapter it will be seen that the term "fibro-vascular," or "fibro-vasal," is open to objections. The author uses the term "vasal-bundles," or "vascular-bundles" (Gefässbündel). The former are, however, in common English use, and are retained here. [ED.]

scope with a low power, a part of the section suitable for further investigation, choosing a fibro-vasal bundle which does not lie too near the periphery, because in this neighbourhood the structure of many bundles is simplified, and fusion with one another occurs. In all cases it is necessary to settle definitely in which direction the periphery of the stem lies, in order that we may know which is the inner and which the outer side of the bundle. The bundle which we select may appear somewhat as in the adjoining Fig. 40.

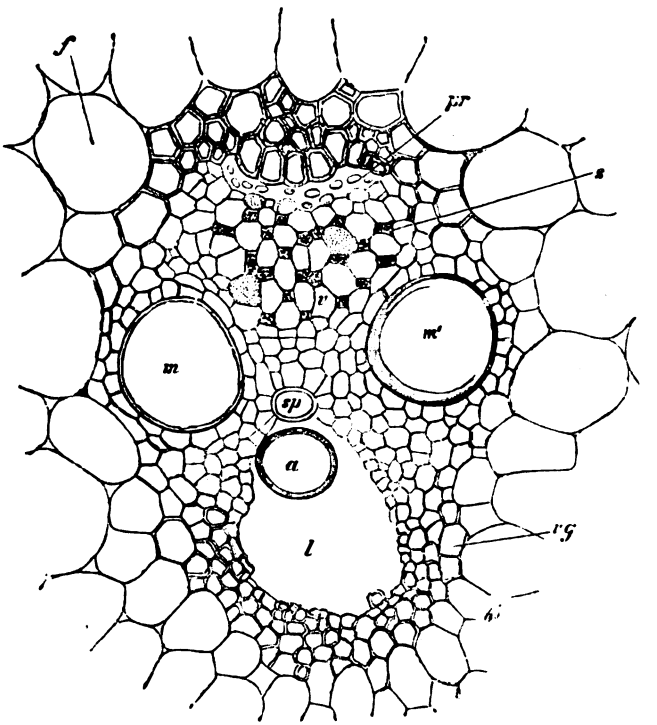


FIG. 40.—Cross-section through a fibro-vasal bundle from the inner part of the stem of *Zea Mais*. *a*, Segment of an annular vessel; *sp*, spiral vessel; *m* and *m'*, pitted ducts; *v*, sieve-tubes; *s*, companion cells; *pr*, crushed elements of the protophloëm; *l*, intercellular passage; *vg*, sheath (× 180).

First to attract our attention is the **Sheath** (*vg*), which surrounds the fibro-vasal bundle, and has become coloured more or less reddish-brown by the chlorzinc iodine. It consists of strongly-thickened and lignified sclerenchyma cells or fibres, and has for that reason stained as indicated above. It is more strongly de-

veloped on the inner and the outer side of the fibro-vasal bundle, but much weaker on its sides. Passing now from the inner side of the bundle towards the outer, we next see an **Intercellular passage** (or intercellular space) (*l*), surrounded by narrow, only slightly-thickened cells, which are nevertheless coloured yellow by the chlorzinc iodine. Into this intercellular space projects a ring (*a*), belonging to an **Annular vessel,** which is usually torn by stretching. The intercellular passage, also, has usually arisen from the breaking down of cells. Such a method of development is indicated by the term *lysigenous*, whereas when it arises only by the separation of the elements of a tissue, the process is *schizogenous*. This torn vessel, together with others which may perhaps also be seen projecting into the intercellular passage, represents the first-formed elements in this part of the fibro-vasal bundle, elements which were developed at a time when the parts of the plant with which we are now concerned were still in process of rapid growth in length. Impinging on the intercellular passage on its outer side, are one or more other vessels. They are recognisable by their cavity, which is larger than that of the neighbouring cells. In the bundle represented in Fig. 40, only one such vessel (*sp*), and that a rather narrow one, is observable in the cross section. These vessels, present to the number of one or more, are, as can be demonstrated only in longitudinal section, thickened in a spiral manner. They are **spiral vessels.** Next, in each half of the bundle, right and left, is a wide cell-cavity (*m*, *m'*). These are two vessels with netted (reticulate) or pitted, rarely spiral thickening. They are the so-called **pitted ducts.** Often in the cavity of these great vessels a ring, or part of one (*m'*), can be seen projecting as a thickening of the wall. This is the relic of a cross partition-wall which, diaphragm-like, was broken through. The cells which surround the two great vessels are reticulately thickened: on their sides turned towards the ground-tissue these vessels are usually, however, bounded directly by the elements of the sheath. The elements lying between the two great vessels show also, in general, the network thickening, and appear as a somewhat darker band joining these vessels. They are usually arranged in regular lines in the direction of the radial axis of the bundle. All the walls of the vessels, and especially those of the two great vessels, are coloured yellow by the chlorzinc iodine. In the two great vessels it happens that this coloration is more intense on that side where they are bounded by the sheath. The

elements between the two large vessels are coloured a somewhat deeper yellow than those surrounding the intercellular space.

The part of the fibro-vasal bundle which we have thus far described is distinguished as the **Wood**, or **Xylem**, or as the "vascular part," and also by the name "**Hadrome**." On practical grounds we prefer the older name of Wood, or Xylem. These terms do not, therefore, involve, as we at once see in this first example, the presence of the strongly-thickened elements on which the common idea of wood is founded. The never-failing element of the wood portion of the fibro-vasal bundle is the vessel, and, therefore, the morphological term formed according to this is the most rational. The selection of the term "Wood," however, simplifies the terminology, and permits the primary part of the bundle, and the secondary growth which we shall hereafter describe, to have corresponding names given to them. For our earliest descriptions, therefore, we must give the preference to this older terminology, in accordance with which many terms still in use have been constructed. In the example studied above we have found, therefore, in the wood portion (the Xylem) of the fibro-vasal bundle, the primary wood, the **Protoxylem**, composed of primary wood-parenchyma and of vessels. In opposition to the wood portion, we must choose for the second part of the fibro-vasal bundle the term **Bast**, or **Phloëm**, against which names we must raise the same objections as to the term wood. In the above example we have similarly a bast portion without the presence of what is usually spoken of as bast. As the sieve-tubes of the bast are never wanting, morphologically the most rational term for it is **Sieve portion**.[2] In contradistinction to Hadrome, the bast portion is also, on physiological grounds, distinguished as **Leptome**. Wood and bast together constitute the Fibro-vasal bundle. As here the wood is in unilateral contact with the bast, these bundles are distinguished, in structure, as **Collateral**. If we wish to include the sheath, which mostly appertains to the ground-tissue, in one technical term with the fibro-vasal bundle, we speak of the whole as the **Fibro-vasal string.*** The physiological considerations which occasion a separation of the fibro-vasal bundle into Hadrome and Leptome, have led to the choice of the term **Mestome** for the entire bundle.[3]

The bast portion of the fibro-vasal bundle which we have under

* This is in all respects a preferable term to the more common one, Fibro-vasal bundle. [ED.]

observation takes on, with chlorzinc iodine a distinct violet coloration: it consists of unlignified elements. Cells with broader, and those with narrower openings appear in regular order. The first are, **Sieve-tubes** (*v*), the latter (*s*) are the companion-cells. Not infrequently the section cuts the cross-wall of a sieve-tube, and this cross-wall appears finely punctate, after the fashion of a sieve, (compare the figure). At the periphery of these elements are always to be seen a number of cells with strongly swollen walls, and cavities almost obliterated (*pr*); these are the sieve-tubes and companion-cells which were first formed, but now have their function suspended; they correspond with the first developed elements of the wood, and in contradistinction with it are distinguished as the primary bast, or **Protophloëm.** With chlorzinc iodine they usually take on a brownish coloration. These cells are bounded by the cells of the sheath, and the innermost of these always are marked by the special width of their cavities. The sclerenchyma cells of the sheath pass over, by means of some intermediate members, into the large-celled parenchymatous ground tissue(*f*). The walls of these large cells of the ground tissue are also, in fully-developed stems, coloured yellow by the chlorzinc iodine, only here and there with a dash of violet. Passing still nearer to the periphery of the stem, we notice that the fibro-vasal bundles are here pressed more closely together, that the intercellular passage disappears from them first of all; in some cases the elements, particularly those of the bast, are reduced, while in all the sheath augments in strength. We notice, even in the inner bundles, that the elements of the sheath are especially thickened and lignified on the inner and outer edges of the bundle. At the sides we see the more strongly thickened and lignified elements only at the sides of the two great vessels. The weaker development of the sheath at the sides of the bast and of the inner portion of the wood facilitates the passage of nutrient fluids between the fibro-vasal bundle and the large-celled ground-tissue. In the fibro-vasal bundles lying more externally, with more strongly developed sheaths, the communication is maintained on both sides of the bast. Lastly, on the most external bundles, with greatly reduced bast, almost sunk between the vessels of the wood, the sheath is proportionally weakened on the outer side of the bast. The communication between the bundles and their environs is provided for in this way in *Zea* (the Maize), and similarly in other cases. Lateral union (anastomosis) of small bundles with large ones is commonly to be seen in the periphery

of the stem, and the reciprocal meeting always takes place laterally at the places where the great vessels lie. Close in upon the epidermis of the stem, is a more or less strongly developed ring of tissue, the elements constituting which appear just like those of the bundle-sheath, and also react clearly with chlorzinc iodine. Such a distinct sheath of tissue bounding the epidermis is distinguished under the name **Hypoderma.** This hypoderma is interrupted only under the spots where lie the stomata. The hypoderma and the sheath of the fibro-vasal bundle have alike to provide for the protection of the thin-walled tissue and for the stability of the entire part of the plant, and are included amongst the elements of the mechanical system,[4] as **Stereïdes,** while the tissue which they constitute forms the system of mechanical tissue, the **Stereome.** In proportion as the stem must be constructed secure against flexion, so must the mechanical appliance, the stereome, be removed as far as possible towards the periphery. The crowded peripheral fibro-vasal bundles, provided alike on the side of the bast and the wood with a strong cover of sclerenchyma, represent here a system of complex upright girders. The sheaths of sclerenchyma are the ties, the fibro-vasal bundles themselves are the "filling." The hypodermal hollow cylinder of sclerenchyma strengthens this action, even when, as in this case, not strongly developed. This hollow cylinder is mechanically to be considered as a combination of numerous "ties," arranged in a circle.*

* I have felt unable to give a satisfactory translation of the above passage in the text. I propose therefore briefly further to endeavour to illustrate it. Two sets of phenomena have to be mechanically provided for, the one affecting the stem as a whole, the other its separate fibro-vasal bundles. First, as to the stem as a whole. It has considerable weight to bear, its own and that of its leaves. It must therefore be mechanically constructed to resist crushing. It has to bear often considerable lateral strains, from winds. It therefore must also be constructed to resist flexion. In both these respects it can be compared with a pier of a bridge, especially a cylindrical iron pier of a lofty railway bridge. To resist flexion this is made hollow, so as to throw all the strength to the outside; and, to aid in resisting crushing, it may be filled with concrete. Secondly, as to the individual fibro-vasal bundles. The sclerenchyma layers will help in the above purpose; but the bundles, being on the one hand water, and on the other hand food conduits, must be protected mainly from the lateral strains which would tend to crush their elements, make them "collapse," and so cease to functionate. This protection is the main duty of the sheath of sclerenchyma. Its being thickened most on the inner and outer side of the bundles, and taking thus the form largely of two arches concave to each other, makes its structure the most advantageous for its purpose, since the main strains in such a cylindrical stem are radial. To this we may add one more factor: the course of each of the fibro-vasal

Very instructive is it to lay some cross-sections in a solution of soda-corallin. All the lignified elements of the fibro-vasal bundle, and of the ground-tissue, stain in a short time a brilliant coral-red, the non-lignified elements rose-coloured. In the section thus treated, the sclerenchyma cells of the sheath, especially at the inner and outer edges of the bundle, stand out conspicuously, and the walls of the vessels are coloured similarly to the sheath, but somewhat more brownish. The hypodermal ring colours the same as the bundle-sheath.

It is worth while now to make a radial longitudinal section through the stem. To obtain this, take a piece of the stem about $\frac{1}{4}$ or $\frac{1}{3}$ inch long, cut it in two longitudinally through the middle, and take the sections from the cut surface of either half. Do not be satisfied with a single section, as otherwise the chance of obtaining in the preparation a fibro-vasal bundle cut actually median is too slight. Such a median-cut fibro-vasal bundle can be recognised on examination of the section, in that it shows at the same time the bast portion and the annular vessel projecting into the intercellular passage. If the longitudinal section is laid in chlorzinc iodine we can at once readily make out a violet coloration of the bast portion, and the thin-walled cells surrounding the intercellular passage have also a violet colour. The other elements, as we have seen in the cross-section, are distinctly coloured yellow to yellowish-brown. For the rest, we prefer to select, for further study, a section which we have previously stained with soda-corallin (Fig. 41). Here also it is desirable first of all to determine in which direction lies the surface of the stem. As in the cross-section, we pass in our examination from the inner towards the outer side of the bundle. We then see that to the broad cells of the ground-tissue, in outline well-nigh square, succeed narrower ground-tissue cells, and after these follow the narrow cells of the fibro-vasal bundle-sheath (*vg*). These last elements, deeply stained with corallin, show marked elongation, join one another with horizontal or more or less inclined end-walls, and are provided with small, cleft-like, obliquely ascending pits. In their

bundles from its lower to its upper extremity is usually not straight, but in the form of an elongated arch, the concavity outwards, they thus become akin to "struts." If they anastomose, or join together, as they do most beautifully in some cases, the mechanical analogy is still more complete, since they then resemble the network of connecting girders with which all observers of iron bridges are familiar. I select iron bridges for this illustration, for in them, as in nature, the smallest amount of material is made to go the greatest possible way. [Ed.]

interior is to be found a peripheral layer of protoplasm of very reduced dimensions, and in each a small nucleus. We have here to do with elongated sclerenchyma cells. To the cells of the sheath succeeds the intercellular passage, and we can determine that it follows without interruption the whole length of the bundle. Thin sections are often entirely broken into two parts by this passage. It is surrounded by thin-walled cells, which are far shorter than those of the sheath, have more cell-contents, end in horizontal walls, and can be described as **primary wood-parenchyma.**

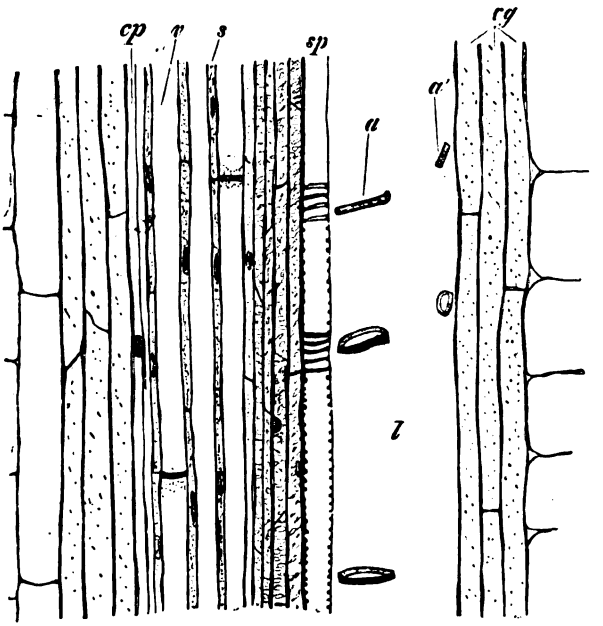


FIG. 41.—Longitudinal radial section through a fibro-vasal bundle in the stem of *Zea Mais*. *a* and *a'*, segments of an annular vessel; *sp*, spiral vessel; *v*, sieve-tube; *s*, companion-cells; *cp*, protophloëm; *l*, air passage (intercellular passage); *vg*, sheath (× 180).

Into the intercellular passage project usually isolated rings; they are attached to the outer side of the intercellular passage, *i.e.* to that side nearest the periphery of the stem. They arise from an annular vessel torn during the elongation of the internode. Other smaller isolated rings may also often be seen clinging to this or the other side of the intercellular passage (*a*). Collectively they represent the remnants of the elements of the protoxylem. Im-

pinging on the larger rings outwardly are one or several broader or narrower spiral vessels. In the case represented in Fig. 41, only one such was present, and that a pretty narrow one (*sp*). Further succeed comparatively short primary wood-parenchyma cells, with pitted and partially reticulately thickened walls. These cells are somewhat more strongly thickened than are those by the intercellular passage. Thus we arrive at the bast portion, recognisable in the corallin preparation by some thick rose-red coloured cross-walls, the **sieve-plates** of the sieve-tubes (*v*). These sieve-plates are highly refractive; and stronger magnification shows that they are pierced by fine pores, after the fashion of a sieve, and that on one side, seldom on both, is collected a highly refractive plug of "slime." In the periphery of the bast (at *pr*), where in the cross-section were visible the swollen cell-walls of the protophloëm elements, a specially beautifully-coloured cross-plate shows up. This is a sieve-plate covered with **Callus**, the structure of which we shall further study hereafter on other more favourable objects. The callus-plate extracts the corallin with special avidity, and therefore stands out so sharply stained.[5] By the side of the sieve-tubes can be distinguished the companion-cells. They are narrower and shorter than the sieve-tubes, and have besides other richer contents, and a readily visible nucleus, for which we look in vain in the sieve-tubes. Cells of the sheath again bound the fibro-vasal bundle. Their end walls are here in part so strongly inclined that we can speak of them as **sclerenchyma-fibres.** The innermost cells of the sheath have, as the cross-section has already shown, a comparatively broad cavity. Starch grains are not found in the fibro-vasal bundle; here, however, they are wanting in the cells of the ground tissue also. All the cells of the fibro-vasal bundle and of the ground tissue, with the exception of the cells forming the vessels, and of the sieve-tubes, contain nuclei.—It is clear that such a median longitudinal section of the bundle as is described above can show neither of the two great vessels. If the section be not exactly median, or not quite thin, such may show by deeper focussing, but it is then however very indistinct. In order to study the longitudinal section of one of the great vessels, we must look for a section which cuts the fibro-vasal bundle laterally. We shall then see that the great vessel is obliquely pitted, and more seldom spirally thickened. In these pitted ducts the thickened parts form a network. The pits broaden out at their bottom, but are however only unilaterally "bordered," in that the corre-

sponding pit of the adjoining cell of the wood-parenchyma is wanting in a "border." These cells, too, are far less thickened than the vessels. The diaphragms, or cross-walls, of the great ducts quickly attract attention in the longitudinal section. They show a doubly formed ring, which besides only projects a slight distance into the cavity of the duct. These rings originated in a thickening of the outer edges of the cross-walls, the inner unthickened part of which was afterwards dissolved (resorbed). From the number of the diaphragms we can therefore draw a conclusion about the number and size of the cells of which the duct is composed. Corresponding with the diaphragms, the vessel shows slight constrictions on its outer side.

It may be of interest to put up some well-chosen cross- and longitudinal sections of the fibro-vasal bundle as permanent preparations. The colours obtained by means of chlorzinc iodine and corallin are not permanent in such preparations; but lasting colours can be given by means of saffranin or iodine-green. Very instructive double staining can be effected if the section is first placed for a short time in iodine-green, and then somewhat longer in Grenacher's alum-carmine[6] or for a similar time in Hoyer's ammonio-acetic carmine; instantaneous double staining can be obtained by means of picric-nigrosine, or picric-aniline-blue. In this way the alum-carmine, the ammonio-acetic carmine, the nigrosine and aniline-blue, respectively stain the unlignified, the iodine and the picric acid the lignified tissues in the preparation. The cell-contents take the colour of the carmine, the nigrosine, or aniline-blue respectively. The preparation can be put up in glycerine jelly or in glycerine. In the latter case, the edge of the cover-glass must be hermetically closed. For this purpose we remove with blotting-paper any glycerine that may have flowed from under the edge of the cover-glass, and surround this edge with a solution of Canada-balsam * in turpentine, benzole, or chloroform, made as thick as syrup. The operation is best performed by means of a glass rod, about the thickness of a thick match, from which the superfluous Canada-balsam is first allowed to run. Gold-size is not suited for closing glycerine preparations, as it will not cling to a glass surface moistened with

* Of these the solution in turpentine is best, as it does not become brittle when dry; otherwise a jerk may make the cover-glass spring. The solution can be kept in a bottle with a bell-shaped external ground cap, to keep out the air. [Ed.]

glycerine. It is however highly desirable to cover the Canada-balsam with gold-size after it has become quite firm. For this purpose it is best not to use the gold-size too thickly, but to put on a second layer. In this operation a fine camel-hair pencil should be used.[a]

Instead of the stem of *Zea Mais*, in case this plant is not at our disposal, can be taken, with very similar results, the stem of *Avena sativa* (the Oat), or that of some other grass.

Now take cross and longitudinal section of a fully-developed leaf of *Iris florentina* preserved in alcohol. Here also the preference is given to alcohol material, because it is more easy to obtain good sections, it contains no air, and besides that, the cell-contents are fixed, so that we can more readily obtain information about them. The section-cutting will be facilitated if the material is previously laid in a mixture of alcohol and glycerine. Lay the sections for some hours in alcoholic borax-carmine; then treat them for a short time with iodine-green. The cell-contents have taken up carmine, which in the form of borax-carmine does not stain the cell-walls; on the other hand the lignified walls are stained green with iodine-green. The vessels, at least, appear stained, and usually also the outer elements of the sheath, *i.e.* those impinging on the bast of the bundle. Besides this, a group of elements with swollen walls, the protophloëm, stand out in the outer region of the bast by their blue coloration. We will therefore commence with the study of such a preparation, from which the Fig. 42 is constructed. In this figure all the cells which are especially rich in contents, and therefore are conspicuous from their red coloration, have their interior shaded. The green-coloured walls of the vessels are, on the other hand, represented darker in the figure, while the group of protophloëm elements coloured blue are left clear. The thickened elements of the ground-tissue bounding the bast, when the section is taken through the base of the leaf, are yet unlignified, and therefore remain unstained. To rapidly stain a section, it can be treated with iodine-green alone; the staining of the cell-contents red as here described is then absent. If the iodine-green should stain only the lignified cell-walls, the exact time for staining must be carefully watched. We proceed in the examination from the wood towards the bast, and therefore from the upper surface of the leaf, turned to the interior, towards the lower surface, turned outwardly. We first determine that the number of vessels in the wood is pretty considerable, and that

[a] See note on page 99*a*.

their width diminishes towards the bast. The vessels directly impinge upon one another, or else are separated by the slightly thickened comparatively narrow cells, with abundant cell-contents, of the primary wood-parenchyma. Similar cells also surround the vessels on the flanks of the bundle, and separate them from the ground-tissue. At the inner margin of the wood are always to be seen some crushed elements, proto-xylem elements (*ss*), whose walls are stained like those of the vessels. The bast shows again an alternation of larger and smaller cells; the contrast is here however not so striking, nor is the regularity so great, as in *Zea*. The cells with broader cavities are the sieve-tubes, the smaller ones, marked out by their abundant cell-contents, the companion-cells. In the outer region of the bast lie the crushed protophloëm elements (*pr*), to which we have already referred, whose function is lost, and which are provided with swollen walls

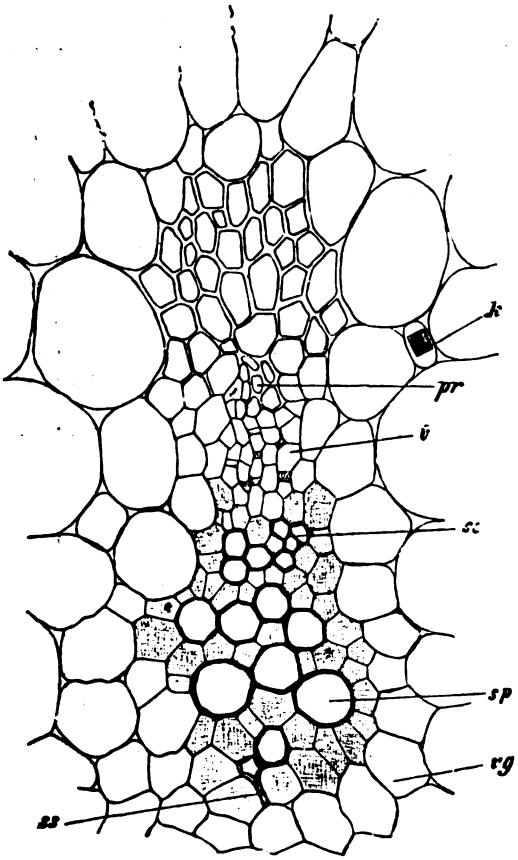


FIG. 42.—Cross-section of a fibro-vasal bundle from the leaf of *Iris florentina*. With dark contour are the vessels; the cells of the bundle which are rich in contents are shaded. *ss*, crushed spiral vessels; *sp*, broader spiral vessels; *sc*, scalariform vessels; *v*, sieve-tubes between which are the narrow companion-cells; *pr*, crushed protophloëm elements; *vg*, sheath with wavy radial walls; *k*, section through a crystal (× 240).

more or less deeply stained blue. This outer bast portion is enclosed by the strongly thickened sclerenchyma of the sheath, which supports the fibro-vasal bundle with a more or less powerful string. Around the remainder of the fibro-vasal bundle a clearly marked sheath is wanting; yet it can be determined that the cells of the ground-tissue nearest to the fibro-vasal bundle are smaller, and that they join together without a break. At the flanks of the bundle these small cells are represented by but a single layer, at the inner boundary of the vascular part, on the contrary, by several layers; here also the walls of some of these cells are well stained blue. The transition to the larger cells of the ground-tissue, which have intercellular spaces filled with air between them, takes place by intermediate forms.

By a scrutiny of the tissue in the neighbourhood of the fibro-vasal bundle it will be seen that single small cells, between the large cells of the ground-tissue, contain a highly refractive crystal (Fig. 42 *k*). It offers itself to us either in cross-section, or in end view; as to its form in longitudinal section we can readily inform ourselves.

Similar crystals, contained in narrow cells, can likewise be seen here and there interposed amongst the larger cells of the general tissue of the leaf. Preparations which it is desired permanently to preserve can be best stained with very dilute watery safranin, which must be allowed to work for only a short time.

In order to control the results hitherto obtained, take some cross-sections also from a fresh leaf. We then determine that the large cells of the ground-tissue in the outer parts of the leaf contain chlorophyll-grains, while the cells included in the fibro-vasal bundle sheath are however wanting in chlorophyll. In fresh preparations the vessels are filled with air, whence the structures are less clear than in alcohol material.

A longitudinal section through the leaf, cutting through the middle of a bundle, shows us at the inner limits of this bundle the strongly extended, partly crushed spiral vessels, which we already saw in cross-section at *ss*, and distinguished as elements of the protoxylem, *i.e.* as the first-developed elements of the wood portion of the bundle. Following are broader more closely wound spiral vessels, and then again scalariform vessels with narrow cavity. In the bast the sieve-plates show clearly only in corallin preparations. Further outwards the sclerenchyma fibres are recognised by their strong thickening, their notable length, and pointed ends.

As the crystals are directed parallel to the long axis of the leaf, they show in profile in longitudinal sections (Fig. 43, *A*, *D*). They lie in elongated cells of the ground-tissue, which are only a little larger than the crystals themselves. These cells contain no chlorophyll, while the neighbouring cells usually contain chlorophyll. The crystals in question dissolve readily in hydrochloric acid without evolution of gas, whence we readily conclude that they consist of oxalate of lime. All the crystals occurring here have an elongated prismatic form, and belong to the monoclinic system; most of them appear geminate (twin crystals), (*D*).

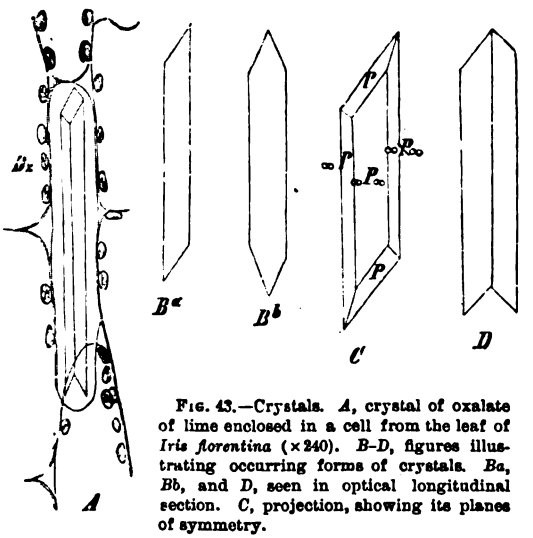


FIG. 43.—Crystals. *A*, crystal of oxalate of lime enclosed in a cell from the leaf of *Iris florentina* (×240). *B–D*, figures illustrating occurring forms of crystals. *Ba*, *Bb*, and *D*, seen in optical longitudinal section. *C*, projection, showing its planes of symmetry.

The contents of the crystallogenous cells are not stained with corallin.

The fibro-vasal bundles of the Monocotyledons, if we exclude immaterial modifications, reductions, and combinations, are constructed upon the type of the two cases we have thus investigated, and we can therefore abstain from further study of these bundles.

Closed fibro-vasal bundles are not capable of increase in thickness, and therefore where such occurs in the Monocotyledons, it cannot be brought about through the medium of the fibro-vasal bundles. This increase of thickness results from the action of of a **Cambium-ring** which is found outside the fibro-vasal bundles, and is confined to the families of Dracæneæ and Aloïneæ *i.e.*, the so-called "Arborescent Liliaceæ" and the Dioscoreæ.

For this purpose we select as a favourable object of study the plant so commonly cultivated in gardens and nurseries as *Dracæna rubra* (more properly, *Cordyline rubra*). The plant must be sacrificed for the purpose of the investigation. Examine

first with the naked eye the stem cut across; we shall notice inside the brown cork-layer the green soft cortex, somewhere about $\frac{1}{15}$ inch thick, towards which the yellowish hard tissue of the stem is limited by less sharply-defined bounds. At this boundary lies the cambium ring. In the yellowish tissue of the stem it is moreover distinguished from the cylindrical centre by its lighter coloration.

We now submit a cross-section to microscopical examination, and first with weak magnification (Fig. 44). We then see, first, in the central portion of the stem, a ground-tissue composed of rounded cells (*m*), in which are irregularly scattered isolated circular or elliptic fibro-vasal bundles. Outward from a definite position (the inner *f''*) the bundles are more numerous, elongated in radial direction, and press so closely together that they appear separated only by comparatively thin streaks of ground-tissue. In these latter the cells are more strongly thickened, coarsely pitted, more or less elongated in the direction of the radius, and clearly arranged in radial rows with often wavy course. Further on, we come to the boundary between the yellowish inner tissue and the green cortex (*c*). We find

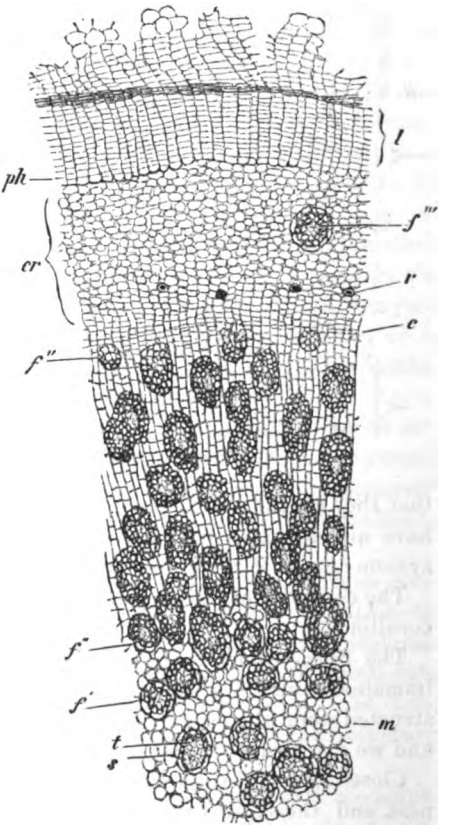


FIG. 44.—*Dracæna* (*Cordyline*) *rubra*. Cross-section through the stem. *f*, fibro-vasal bundles, *f'* being primary, *f''* secondary, and *f'''* leaf-bundles; *m*, unlignified elements of the ground tissue; *s*, lignified elements of the ground tissue, sheathing the fibro-vasal bundles; *t*, tracheïdes; *c*, cambium ring; *cr*, cortex; *l*, cork; *ph*, cork-cambium; *r*, bundles of raphides (×30).

here a zone composed of flattened thin-walled cells, strictly arranged in radial rows. It is the **Cambium-ring**, which provides for the increase in thickness of the stem. It belongs apparently to the ground-tissue. Its most flattened cells lie in the middle of its cross-section. In it is found the real **initial-layer**, the cells of which, in course of constant division, produce on their inner, and to a smaller extent their outer, side new elements. These divisions result from the formation of tangential walls, and produce therefore radially arranged cell-rows, which from time to time are made double by the formation of radially directed walls. Embedded in the growing tissue resulting from this cambium-ring are numerous fibro-vasal bundles in all stages of development. The youngest consist of a group of thin-walled cells, the oldest are already perfect at their inner portion, while the thin-walled outer portion *i.e.*, the portion towards the periphery, are still immersed in the cambium-ring and in course of development. From the position where the fibro-vasal bundles appear crowded together, and the cells lying between them have acquired a radial arrangement, the tissue is **Secondary**, produced by the activity of the cambium-ring. The **Cortex** (*cr*), succeeding outwardly to the cambium-ring, consists of rounded cells. Between these, especially in the inner part of the cortex, occur single cells, in which lie fine needle-like crystals, in each cell closely packed together into a bundle (*r*). These are the bundles of so-called **Raphides**, consisting of oxalate of lime. They are here seen in end view. Individual raphides-containing cells are sure to be opened by the razor in cutting, and the fine needles will therefore be found lying scattered over the section. The rest of the cortical cells contain chlorophyll-grains. In the cortex are also visible single round cross-sections of bundles (*f'''*) which are passing outwards into the leaves. There succeeds a thick layer of thin-walled, colourless cells, arranged radially (*l*), which on its outer side passes over into a brown, less regular, tissue. This is the **Cork-layer**, consisting of growing colourless cork-tissue in its more internal, but of old irregularly elongated and discoloured cork-tissue in its outer parts.

Especially instructive are cross-sections treated with corallin. The fibro-vasal bundles are thereby sharply defined. The corallin also always colours deeply the lignified cells of the secondary ground-tissue, but of another shade. The unlignified cell-walls appear pale rose-coloured. In the cortex, the cells containing

raphides appear now filled with clear coral-red to orange-coloured contents. We easily determine, by the aid of this coloration, that the raphides lie embedded in a homogeneous **Slime** [or **Mucilage**], which accumulates the corallin. Besides the power, which it shares with aniline-blue, of colouring the Callus of the sieve-plates, corallin has the specific property of staining **Vegetable Slime** or Mucilage. If we lay the longitudinal section of *Dracœna*, stained with corallin, in alcohol, and, moreover, allow this last to boil, the slime remains none the less stained. From this we can conclude that, as far as knowledge goes, it consists of a **Starch-slime**, while the slime the result of the degradation of cellulose is decolorized sometimes even in cold, but at any rate in boiling alcohol.[7] **Gums** are not stained by corallin; mixtures of slime and gum (**Gum-slime**), according to the proportions. On the other side, we can determine that watery solution of nigrosine does not stain the slime here present, even after long-continued action; while it stains the slime of *Rumex* (see page 79).

With this insight into the cross-section, which indeed suffices to give us information on the phenomena of increase in thickness, we will be content, and in this case abstain from the study of the further peculiarities, as also of the longitudinal section of the stem.

REMARKS ON CHAPTER VIII.

[1] On the fibro-vasal bundle compare, above all, De Bary, *Comparative Anatomy* (Eng. trans. 1884), especially in chapter viii., where will be found the entire older literature. Numerous more recent researches into the morphology of the fibro-vasal bundle have not since then received collective treatment. This has, on the other hand, been done, as far as the anatomo-physiological works are concerned, by G. Haberlandt, in *Encyklopädie der Naturwissenschaften,—Handbuch der Botanik*, Bd. II. p. 593.

[2] The terms vascular-part (Gefässtheil), and sieve-part (Siebtheil), introduced by De Bary, *Comp. Anat.* chap. viii. [In the English translation of this work the terms are replaced by "xylem" and "phloëm" respectively.]

[3] Compare Haberlandt, *Die Entwicklungsgeschichte des mech. Gewebesystems der Pflanzen* (The Development of the Mechanical System of Plants).

[4] Schwendener, *Das mechan. Princip. im anat. Bau der Monocotylen* (The Mechanical Principle in the Anatomical Structure of Monocotyledons).

[5] This staining-fluid introduced by Szyszylowicz. Compare *Bot. Centralb.* Bd. XII. p. 138.

[6] Compare Tangl, *Jahrb. für wiss. Botanik.* Bd XII. p. 170.

[7] Compare Szyszylowicz, as above.

[*Note to page* 93.]

• Of the above-mentioned stains, that with safranin is the only one which s quite permanent. As it gives very beautifully differentiated figures, it can be pecially recommended. Permanent double staining can be obtained by the use f methyl-violet. The sections are first treated for about five minutes with a ully concentrated alcoholic solution of methyl-violet, then so far decolorised n alcohol that the unlignified membranes appear only feebly stained, then ransferred to water for some minutes, and from this into one of the carmine olutions already mentioned. Some control is needed in order to see when the taining has been rightly carried out, and the decolorising is best effected on an bject slide under the low power of the microscope. If the tiny white earthenware saucers sold for paint boxes are used for staining and decolorising, the white background much facilitates the process to the naked eye. Very effective double staining can likewise be obtained by first staining with logwood, then with magenta in 50 per cent. alcohol, and then carefully removing this latter colour by means of alcohol (in which it can be completely dissolved out) until it is left only in the lignified and cuticularised membranes.—Hoyer's mounting fluid is very useful for permanent preparations, and, like glycerine jelly, needs no further closing.

For the transfer of large sections (the edges of which are liable to curl over) from one fluid to another, "section lifters" made of thin platinum, with a wooden handle, will be found useful; or copper wire with one end beaten out as recommended in the Introduction.

CHAPTER IX.

OPEN COLLATERAL VASCULAR BUNDLES.

Material Wanted.

Runners of the Creeping Buttercup (*Ranunculus repens*). Fresh, or in alcohol.
Stems of the Celandine (*Chelidonium majus*). In alcohol.
Roots of *Scorzonera*, or of Dandelion. Fresh, or in alcohol.
Stems of *Aristolochia Sipho*, $\frac{1}{8}$ to $\frac{1}{5}$, and $\frac{2}{5}$ inch thick. In alcohol.

As a first example for the study of **open collateral vascular bundles**, as they are peculiar to the Dicotyledons, we select the creeping stems (runners) of *Ranunculus repens* (the creeping Buttercup). We cut through the older, fully developed parts, and stain the sections with safranin, in order to facilitate our task. The cross-section shows that the vascular bundles are completely isolated from one another, but arranged in a single circle in the stem. The ground-tissue consists of rounded cells, which become smaller towards the periphery of the stem, contain chlorophyll-grains, and have between them large intercellular spaces. The epidermis forms the surface of the stems; in the interior, through the stretching apart and destruction of the cells, the stem is hollow. The vascular bundles give throughout the same impression as those of the Monocotyledons; the same parts are recognisable in the same order. The xylem portion consists of vessels and wood parenchyma. The vessels nearest to the inner side of the bundle have not taken up much stain; they are annular and spiral vessels belonging to the protoxylem. To these succeed broader spiral vessels (Fig. 45 *s*) and pitted ducts. These latter appear somewhat angular, and their walls betray the bordered pits. The more lateral pitted ducts are broader, those in the medium portion of the bundle, on the other hand, smaller. Between and around the vessels, especially at the inner edge of the bundle, lies thin-walled wood-parenchyma. Between the narrow median pitted ducts are, in general, only isolated wood-parenchyma cells, recognisable by their contents. All the vessels are

stained brownish-red in the safranin. In the bast is again very visible the alternation of larger sieve-tubes (*v*) and smaller companion-cells. In the periphery of the bast can be recognised the narrow cavities of the protophlöem elements. The bast is, however, separated from the wood by a multilamellar layer of cells arranged radially. These cells remain in the **cambial** state. Here

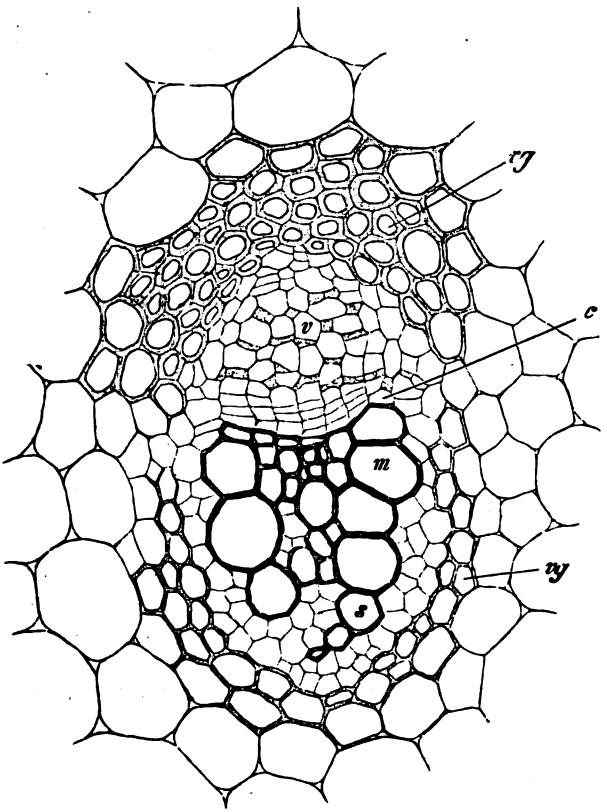


FIG. 45.—Cross-section through a fibro-vasal bundle from the runner of *Ranunculus repens*. *s*, spiral vessels; *m*, vessel with bordered pits; *c*, cambium; *v*, sieve-tubes; *vg*, sheath (× 180).

for the first time we make the acquaintance of such a **cambium**, and it causes these bundles to be ranked as **open bundles**, that is, those which are capable of further development through the activity of their cambium. Such bundles are not found amongst Monocotyledons, but are proper to almost all Dicotyledons. The

cambium of the vascular bundle of *Ranunculus repens* does not, however, enter into renewed activity, so that secondary thickening, such as we shall find later on in this chapter, is not brought about. The vascular bundles are surrounded by a sheath of sclerenchyma-like elements, which are stained a beautiful bright-red in safranin. The sclerenchymatous elements are specially numerous on the bast side, and are also more strongly thickened; nevertheless the bast itself is separated from them by thin-walled cells. The sclerenchyma fibres are wanting on the flanks of the bundle, marking the limits between the wood and the bast. There the sheath becomes entirely parenchymatous, its elements are unthickened and unlignified, and so form the "places of passage," which provide an easy communication between the vascular bundles and the surrounding ground-tissue. The layer of ground-tissue succeeding to the sheath is distinguished by the richness in starch contents of its chlorophyll-grains, and forms a so-called **starch-sheath**. This latter is specially noticeable on the bast side and the flanks of the bundle, while it becomes unrecognisable on the inner edge. On the bast side its cells not infrequently contain a red cell-sap. In the longitudinal section we can readily determine the presence of annular, spiral, and pitted vessels [pitted ducts], and between them elongated cells of the primary wood-parenchyma; then follow thin-walled cambium-cells, sieve-tubes, and companion-cells. At both edges we find the thickened elements of the sheath, which join on to one another with more or less oblique, pitted, cross-walls; and at the outer edge are also readily recognisable the elements of the starch-sheath, distinguished by their marked shortness, and the richness of their starch contents.

The vascular bundle of *Chelidonium majus* is so similar to that of *Ranunculus repens*, that the cross-section can be understood without further reference. Here, however, we prefer alcohol material. The wood shows large vessels, closely crowded together, which, in the older parts of the stem, have yellowish walls. The bast is strongly developed; between the two lie the thin-walled radial rows of cells developed during the brief activity of the cambium. Only the stronger bundles have a string of strongly thickened elongated sclerenchyma-cells recognisable at the outer edge of the bast. In older parts of the stem these cells likewise assume a yellow colour. For the rest, the vascular bundle is sheathed by ground-tissue cells, which do not differ from those

farther removed, but are not separated by intercellular spaces. These elements are usually distinguished by the presence of numerous chlorophyll-grains, and by abundance of starch. These starch-containing elements are specially represented around the vascular or wood portion of the bundle. The large-celled pith very early becomes hollow. The mechanical stability of the stem is provided for by a ring of elongated, parenchymatous, strongly thickened and lignified, yellow-coloured elements, which surround the whole stem, and are separated from the bundles by the chlorophyll-containing layer of cells. To this ring succeed two layers of narrow cells, of which the inner contain chlorophyll, the outer are collenchymatously thickened, and their walls appear white. The epidermis comes next to this outer layer. In and near the bundle, however, we meet, for the first time, a new element—the **milk-tubes** [**latex-tubes, laticiferous-tubes.**] In the bast portion of the fibro-vasal bundles, and also near the inner limits of the wood, but especially numerous at the flanks and near the outer edge of the string of sclerenchyma, and single examples also in the remoter ground-tissue between the fibro-vasal bundles, we notice cells with dark-brown contents. These contents consist of the orange-red **latex** of *Chelidonium*, coagulated in alcohol. The cells in question are so striking that it is impossible to overlook them. They are all thin-walled, even those which are inserted within the outer edge of the string of sclerenchyma; they are not even distinguished by any special form. The latex-tubes can be found very easily also in radial longitudinal sections.* They are recognised at once by their yellowish-brown contents. They present the appearance here of long tubes running thereabouts parallel to the long axis of the stem. Without difficulty the existence of cross-walls in these tubes can be determined. These cross-walls are more or less clearly pierced in their centres by one or more pores; they are entirely wanting, also, here and there, where we should expect to find them. In not exactly rare cases, one or another of the vessels in the fibro-vasal bundle shows itself to be full of coagulated latex. Lateral communications of the latex-tubes cannot be observed in *Chelidonium*.

* To obtain radial longitudinal sections, take a piece of the stem about ¼-inch long, cut it in half down the middle, and take sections from the cut surface. If the half-stem is too thin to hold readily, stick the point of a needle (in a holder) through it from side to side, and parallel with the cut surface; then, laying the needle flat on the left-hand index-finger, cut the sections. [Ed.]

Anastomosing **latex-vessels (laticiferous vessels)** can, however, be found in the Poppies (*Papaveraceæ*), the Bellworts (*Campanulaceæ*), and in the ligulifloral section of the Composites (*Cichoriaceæ*), as, *e.g.* in the Dandelion. Of these we can select as an example, the garden *Scorzonera* (*S. hispanica*), not infrequently grown in kitchen-gardens for its parsnip-like roots. Tangential sections, taken from the external part of the root, a short distance below the exterior, if treated as described above, will show in the bast portion of the fibro-vasal bundles an extensive network of latex-vessels filled with their very granular contents, shown in Fig. 45*.

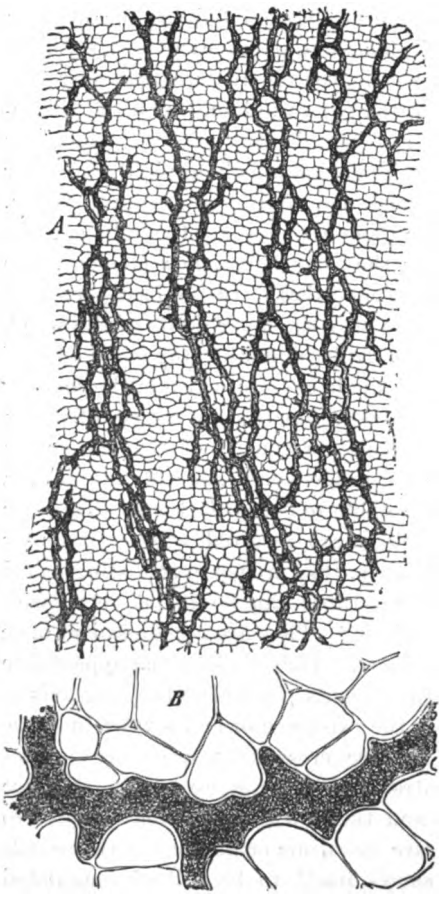


Fig. 45*.—Latex vessels in the bast of *Scorzonera hispanica*, tangential section. *B*, a portion of *A*, more highly magnified. (After Sachs.)

An extraordinarily favourable object for the study of the growth in thickness of the dicotyledons is *Aristolochia Sipho*, a hardy deciduous climbing plant not infrequently cultivated in England, and material for the investigation of which will therefore be probably not difficult to obtain. It is desirable to collect it at the end of June. Our description will be made from fresh material, but, with the exception of the green colour, will serve also for alcohol material. Take first a cross-section, through a twig about $\frac{1}{4}$ or $\frac{1}{6}$ inch in thickness. This section (Fig. 45**)

shows with a lens an internal large-celled pith (*m*), around this a belt of isolated vascular bundles (*f.v.*), round this further a continuous white ring (*sk*), then green cortical tissue (*c*), and finally a yellowish-green peripheral rind (*cl*). With a low power under the microscope we can determine that the pith consists of round, large cells, in part filled with air. In the vascular bundle the wood (*vl*) appears darker, pierced with holes which are the vessels; then follows the cambium zone (*fc*), composed of narrow, radially arranged, clear cells, and then the bast (*cb*), which appears somewhat clear, but without the regular arrangement shown by the cambium zone. Each bundle is bordered, especially in its outer part, by a parenchymatous tissue, containing chlorophyll-grains to some extent, perhaps also reserve food materials. The white ring, succeeding outwardly to these, is composed of strongly thickened sclerenchyma-cells; between the fibro-vasal bundles it projects inwards in a somewhat wedge-like fashion. Impinging on the ring towards the exterior is a tissue containing chlorophyll, the innermost layer of which, bounding upon the sclerenchyma ring, is marked by its richness in starch, and belongs to the category of so-called "**starch-sheaths**." Such starch-sheaths resemble in position the "endodermis" of roots, which we shall hereafter study, and form the innermost layer of the primary cortex. The whole axial portion, thus surrounded by the primary cortex, is known as the *central cylinder*, and its peripheral tissue, separating the vascular bundles from the cortex, and which consists in this case of parenchyma and the sclerenchyma cylinder, is called the *pericycle* (*pc*). After treatment with iodine this starch-sheath stands out very clearly. There follows a tissue,

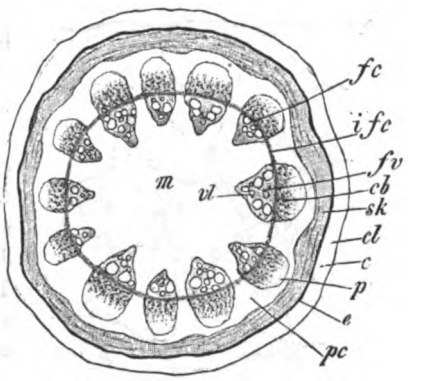


FIG. 45**.—Cross-section through a twig of *Aristolochia Sipho*, about ¼ inch in thickness. *m*, Pith; *fv*. vascular bundles, of which *vl* is the wood, and *cb* the bast portion; *fc*, fascicular cambium; *ifc*, interfascicular cambium; *p*, primary bast parenchyma on the outer side of the bast, which effects a transition to the ground-tissue; *pc*, pericycle; *sk*, sclerenchyma ring; *e*, starch-sheath; *c*, green cortex; *cl*, collenchyma (×9).

likewise chlorophyll-containing and with narrow cavities, with glistening cell-walls more strongly thickened in the corners, in which, by this last peculiarity, we recognise **collenchyma.** [Compare Fig. 45***, showing collenchyma-cells from the leaf-stalk of a *Begonia*.] Outside all is found the epidermis. The collenchyma ring is, however, traversed by certain dark green prolongations of the inner cortical tissue, which extend up to the stomata.—This general information will suffice, and we will now turn to the study of a single bundle. This can only be on very thin sections. Such we prepare with care from the alcohol material, which, in order to be able to cut it better, we have previously placed for 24 hours in a mixture of half alcohol and half glycerine. These sections also are stained by a longer action of safranin. The figure of a vascular bundle in course of development from a twig of the current year, placed in alcohol about the beginning of June, appears as in Fig. 46. The vascular bundle begins at its inner margin with thin-walled primary wood-parenchyma (*p*), in which are enclosed narrow vessels (the protoxylem) gradually becoming broader as we pass towards the exterior. The primary wood-parenchyma becomes at the same time thicker walled. This applies still more to the vessels, while the space between is occupied by still more strongly thickened **tracheïdes** with bordered pits (*m'*). These are not broader than the wood-parenchyma elements lying between them, but are distinguished from them by more strongly thickened walls, and the absence of contents. The fully developed vessels and tracheïdes, as well as the thick-walled wood-parenchyma, stain an intense red in the safranin, the thin-walled wood-parenchyma, like the ground tissue, only a bright brown colour, against which the innermost vessels therefore stand out very clearly. We can see without

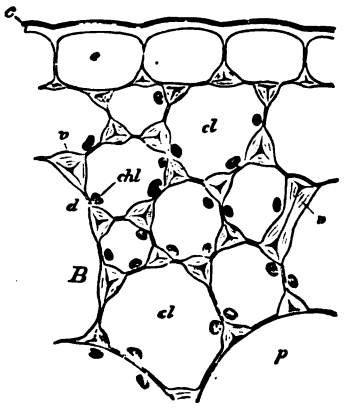


FIG. 45***.—From a transverse section of a leaf-stalk of a *Begonia*, showing the collenchyma-cells, *cl*, underlying the epidermis, *e*; the collenchyma-cells contain a few chlorophyll-grains, *chl*; *p*, is an internal parenchyma-cell; *c*, the cuticle. These collenchyma thickenings are capable of greatly swelling. (After Sachs, × 550.)

difficulty that the innermost, and at the same time smallest, of these are completely crushed. These crushed vessels belong to the protoxylem (*vlp*). The two largest vessels (*m''*) of the vascular bundle represented in the figure are in course of development. Between the two developing vessels lies a young thin-walled tissue,

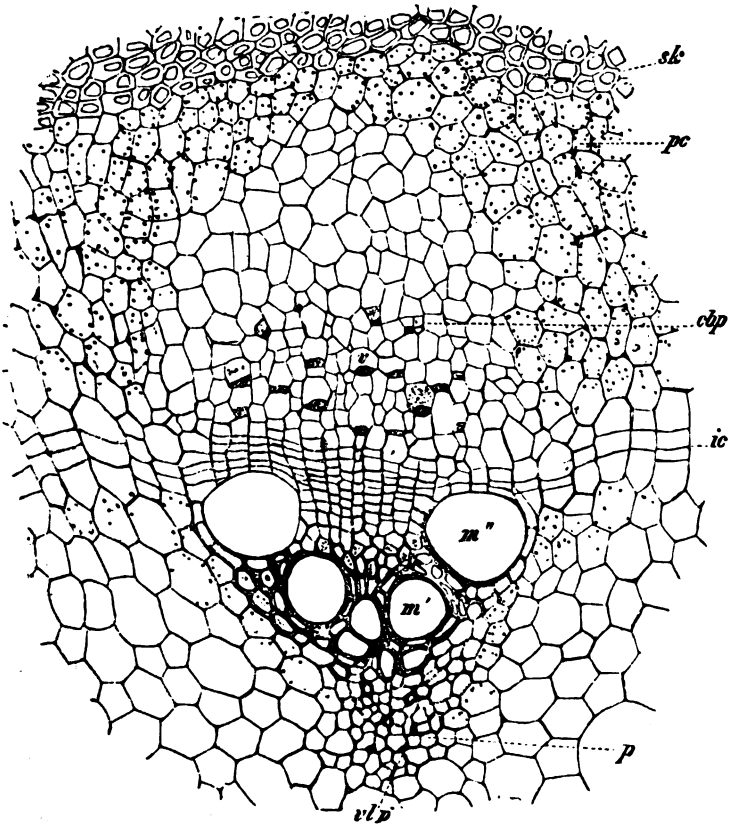


FIG. 46.—Cross-section through a young twig of this year's growth of *Aristolochia Sipho*, showing a fibro-vasal bundle after the cambium has commenced its activity. *p*, parenchymatous elements at the inner extremity of the wood; *vlp*, protoxylem elements; *m'* and *m''*, pitted ducts; *ic*, interfascicular cambium continued into the fascicular cambium, *i.e.* the cambium in the interior of the fibro-vasal bundles; *v*, sieve-tube; *cbp*, protophloëm elements; *pc*, tissue of the pericycle; *sk*, inner part of the ring of sclerenchyma-fibres (×130).

arranged in radial rows, and therefore arising through the activity of the cambium. The **cambium-zone** bounds the outermost limits of the two large vessels; in this a specially flat, but otherwise not

sharply-defined layer of cells, represents the **initial layer.** Succeeding towards the exterior are the thin-walled elements of the bast, the radial arrangement of the inner portion of which also indicates a secondary derivative of the cambium. In this bast are the sieve-tubes (*v*), clearly distinguishable from the companion-cells accompanying them by the abundant contents of these latter. Amongst sieve-tubes and companion-cells there are also scattered cells of the bast parenchyma, smaller in diameter than the sieve-tubes, but broadening towards the limits of the bast, and passing over without sharp delimitation into the wide ground-tissue elements by which the bast is bounded. In the outer part of the bast are narrow sieve-tubes and companion-cells intercalated amongst the broadening parenchymatous elements; they constitute the protophloëm (*cbp*). The bast is separated from the sclerenchyma ring (*sk*) by a large-celled cortical parenchyma, devoid of intercellular spaces. The sclerenchyma ring appears quite as deeply stained as the lignified part of the vascular bundle. Under the pressure of the elements newly developed from the cambium, the protophloëm elements are soon crushed.—The formation of the **interfascicular cambium** is very instructive in such sections. With the commencement of the activity of the cambium in the vascular bundle, the ground-tissue cells impinging laterally upon it (*i.e.* the cambium) have become stretched, and partition walls are formed in them (*ic*). Thus streaks of cambium are developed, passing through the elements of the ground-tissue, which connect together the cambium streaks in the circularly arranged vascular bundles into a continuous cambial ring. As the accompanying Fig. 46 shows, the formation of the interfascicular cambium (*ic*) in *Aristolochia Sipho* is extraordinarily easy to follow, and the original contour of the ground-tissue cells which have been divided can be long recognised. The plates of ground-tissue, separating the individual bundles of the ring, and across which this interfascicular cambium is developed, are the **primary medullary rays.** A structure recognisable as a sheath around the separate vascular bundles of *Aristolochia Sipho* is completely wanting. The ring of sclerenchymatous elements forms a **common sheath** to the whole of the interior tissue of the stem.—A delicate radial longitudinal section, which has cut through pretty near the middle of the vascular bundle, and is stained with corallin, shows, most internally in the bundle, elongated primary wood parenchyma with horizontal end walls,

between them very narrow annular vessels, more or less crushed, then somewhat broader annular vessels, perhaps partially showing transitions to the spiral form; then closely-wound broader spiral vessels, partly showing transitions to the reticulated form; and finally the broadened ducts, with bordered pits. Between these vessels can be seen very elongated tracheïdes, devoid of contents and with bordered pits; single **fibres**, like to the tracheïdes in form, but having pits without borders, and containing starch; thick-walled wood parenchyma, short, with horizontal end walls, and likewise with unbordered pits and starch. The immature ducts appear as broad cylindrical as yet thin-walled cells, separated by horizontal end walls, with abundant peripheral protoplasm, and with nucleus. In the mature duct these contents are no longer observable, and in the place of the complete end walls these **pitted ducts** show only annular projecting diaphragms. The flat ("tabular") cells of the cambium ring show abundant protoplasmic contents, nuclei, and delicate horizontal end dividing walls. The sieve-plates are extraordinarily beautiful. Often they are oblique, and offer to the observer their entire rosy surface with darker shining dots. The side walls of the sieve-tubes are likewise covered with small, finely punctate, rose-coloured sieve-pits, usually elongated in cross direction. In the periphery of the bast is shown in the most striking manner the formation of the **callus-plates**, which cover either both sides of the sieve-plate in similar quantity or preponderatingly on one side only of the sieve-plate, as bright rose-coloured, highly refractive masses, rounded on their free sides, *i.e.* the sides towards the cavities of the constituent cells of the sieve-tube. The small sieve-pits on the side walls also have small callus-plates. Besides the sieve-tubes will be noticed the companion-cells, thickly filled with cell contents, and the broader, shorter cells of the bast parenchyma, usually with starch contents but less protoplasm. The bast is separated from the ring of sclerenchymatous elements by the parenchyma-cells of the ground-tissue. The sclerenchyma-fibres of the ring are very long, pointed at their ends, with their ends interlapping between one another, and are provided with fine pits. Finally we can also determine that the collenchyma-cells adjoining the epidermis are many times as long as broad, and join on to one another with horizontal end-walls.

Let us now continue our observations upon an older stem, about $\frac{1}{2}$-inch thick. First cut it across and examine the cut surface

with the lens. The pith is partially destroyed and considerably diminished, and appears extended obliquely. Through the activity of the cambium, the vascular bundles have undergone considerable increase in mass. The plates of ground-tissue which separate them are elongated to a corresponding degree, similar radial plates of tissue having been added on to them by the cambium *pari passu* with the secondary extension of the vascular bundles themselves. The whole of this tissue, which lies between the pith and the cambium, we describe as the wood, and distinguish therein the xylem bundles from the medullary rays separating them. The xylem bundles consist of the primary vascular portion and the secondary increase; the whole secondary increase in the xylem bundle constitutes the **secondary wood**, which, however, is not separated by a clear line of limitation from the **primary wood**. The pith and the medullary rays appear white, from containing air; the wood is yellowish. The thickest medullary rays, usually ten to twelve in number, end in the pith; these are the "primary" medullary rays, those which from the beginning separated the vascular bundles. Bounding the pith are the oldest wood portions of the vascular bundles. Since the broad-cavitied ducts are wanting there, they show as a denser, darker-coloured ring, penetrated by the primary medullary rays. After this follow the concentric annual rings. The breadth of the openings of the ducts increases during the first year's growth till a definite maximum diameter is attained; it then again diminishes. The limits of the yearly rings are clearly marked by the broad openings of the ducts, in that after the wood of the first year the broadest vessels are only produced at the commencement of the growth in spring. The outer part of the year's ring, that is, the wood which is developed in the late summer of each year, including the first, has no vessels distinguishable with the lens.—Proportionally as the secondary woody body, *i.e.* that developed after the interfascicular cambium has commenced its activity, increases in circuit, new medullary rays are interposed therein, which we can distinguish as medullary rays of the second, third, etc., order, but in general collect under the name of **secondary medullary rays**. The intercalation of new medullary rays takes place here with the greatest regularity. The further we remove our point of view from the centre of the stem, so much the more numerous the medullary rays become, and so much the shorter those newly developed appear. At the outer limit of the woody body we see the cam-

bium ring as a darker circle, which shows also as a delicate line within, *i.e.* in passing through, the medullary rays. On the opposite side to the secondary wood can be seen the clear brown-coloured **secondary bast**, similarly developed from progressive growth. The medullary rays broaden outside the cambium as a result of their subsequent growth in breadth, due to the increase in thickness of the stem. The bast portions are not capable of such a subsequent growth in breadth, and appear, therefore, exteriorly to dwindle and become rounded off. The originally continuous ring of sclerenchyma has been broken into isolated, unequal, olive-green coloured portions; and similarly, also, is the originally continuous and still darker olive-green coloured layer of collenchyma. The protection of the interior has now been undertaken by the **periderm**, which covers the surface of the stem as a brown sheath, and shows clear lamination. The whole of the portion subsequently developed through the activity of the cambium, which includes in itself the secondary bast, and the broadened ends of the medullary rays, is spoken of as **secondary cortex**, in contradistinction to the primary cortex present before the commencement of the increase in thickness. A sharp limit between primary and secondary cortex is here, however, not present.

We next investigate, with stronger magnification and on delicate cross-sections, the structure of the stem described above. The tissue of the pith is found destroyed in the interior, and most of the cells contain air; many cells contain cluster-crystals of oxalate of lime, others starch. Especially rich in starch is the somewhat narrow-cavitied tissue in the exterior of the pith, in which the protoxylem bundles are immersed. This zone we will distinguish as the **medullary sheath.** The xylem bundles have drawn somewhat nearer together into the centre of the stem, crushing together the pith and the inner parts of the primary medullary rays. The same fate has befallen the protoxylem, at least so far as the thin-walled elements are concerned. The vessels formed in the early part of each year show an increase of volume up to the third or fourth yearly ring. From spring towards autumn, in each year's ring, the width of the ducts decreases very rapidly. Shortly before the close of the vegetative period in each year, only very narrow elements are formed. The largest proportion of the wood consists of comparatively narrow and strongly-thickened elements, which have bordered pits and appear devoid of contents, and, therefore, are tracheïdes. They contain

air or water. If contents, such as starch-grains, are seen in them, they have been swept out of neighbouring cells by the razor. Especially surrounding the ducts, but also scattered between the tracheïdes, are somewhat less thickened elements, with shallow pits, protoplasmic contents, and commonly also containing starch; these are wood-parenchyma cells and wood-fibres. The ducts are only provided with bordered pits where they are in contact either with one another or with tracheïdes; where the pit of a duct or of a tracheïde hits upon the pit of a wood-parenchyma cell or wood-fibre, it is only bordered on one side, namely, the side towards the duct or tracheïde, *i.e.* only on this side has the pit its mouth contracted.

The **closing membrane** of such a unilaterally bordered pit is without a central thickening (**Torus**), and, in distinction from closing membranes provided with a torus, colours blue with chlorzinc iodine.[1]

The cells of the medullary rays are elongated radially, their walls comparatively little thickened, and with numerous small pores. They contain starch, air, or cluster-crystals of oxalate of lime. At the outer limits of the ligneous mass we easily recognise the cambium, composed of thin-walled, flattened, radially arranged cells, and on the other side of this the thin-walled elements of the bast. Besides the sieve-tubes and companion-cells are also found in this case starch-containing cells of the bast-parenchyma. The secondary bast, developed through the activity of the cambium, has, therefore, acquired these last additional elements. In exceedingly thin sections, the alternation of non-collapsed layers of cells with collapsed, completely-flattened layers can be followed. The layers of cells which are not collapsed consist of bast-parenchyma cells, for the most part rich in starch, and produced in spring. The flattened layers, on the other hand, consist of the subsequently developed sieve-tubes and companion-cells and scattered bast-parenchyma cells, which are crushed by the growth of the next year. The bands of bast-parenchyma in the older parts of the bast, like the similar elements in those parts which are still active, show up (should they contain starch) very prominently after addition of iodine. Material collected at the beginning of winter is especially rich in starch. The dead parts of the bast become therefore more and more pressed together, and form thus arched white bands, continually becoming thinner and further apart as we pass to the exterior of the bast. The layers

of living bast-parenchyma keep pace, by cell-division, with the increase in thickness of the stem. By the intercalation of new medullary rays the bast undergoes repeated bipartition, and therefore every outer part of the bast always overlaps two inner portions. Outside the rings of bast can be seen the parenchymatous elements of the pericycle, stretched into a thin zone, and then the disconnected fragments of the ring of sclerenchyma-fibres. These fragments are separated by a parenchymatous tissue which has penetrated on both sides between them. That the sclerenchyma fibres of this ring contain protoplasm, and to some extent starch, can be determined even now in the parts of the ring which still remain. The fragments of the starch-sheath can scarcely be recognised, the inner part of the primary cortex contains many cells full of air. The ring of collenchyma is likewise separated into pieces; but here also not merely a rupture results, but in single places tangential extension of its cells ensues, which then commence dividing and so give origin to masses of parenchymatous tissue. The surface of the stem is covered with periderm, which shows wider zones of broad thin-walled, and narrower zones of narrow thick-walled, **cork-cells**, in beautiful alternation. As in the pith and medullary rays, so also in the cortex, are found scattered crystal-masses of oxalate of lime.

The radial longitudinal section shows, in the secondary wood, first the broader and narrower ducts, with bordered pits, and annular diaphragms, the tracheïdes with bordered pits, the less thickened wood-fibres, recognisable by their contents and their shallow pits, and the wood-parenchyma, less thickened than the tracheïdes, and joined into continuous rows or lines. If a medullary ray is cut, its thin-walled cells can be seen proceeding in radial direction, and therefore straight across the section. At the outer limit of the wood we can recognise the cambium-cells, flat (tabular), rich in protoplasmic contents, thin-walled, ending on one another with horizontal walls; then the still active portion of the bast, and then the alternating collapsed, and non-collapsed flattened, elements of the older bast. The laminated periderm in the periphery is very beautifully exhibited. The longitudinal section appears just as does the cross-section; the cells are just as long as broad.—In cutting the wood, the direct course of the medullary rays appears to the naked eye. This arises from the marked length of the internodes, *i.e.* the spaces between the points of origin of the leaves, in which the vascular bundles, like the

medullary rays, preserve their direction unchanged. The tangential longitudinal section of the stem shows under the microscope the medullary rays in the form of broader or narrower streaks, separated from one another by corresponding streaks or layers of the ligneous body of the stem.

If we examine a stem which has been cut in winter, before the commencement of the vegetative period, and laid in alcohol, we notice that the starch has disappeared from the stem. If at the same period a fresh stem be examined, we find, in place of the starch, yellow, strongly refractive oil-drops in the cell. The starch in most woody plants appears to undergo a similar change during the winter. The oil-drops have disappeared in the alcohol material.

As it is always a matter of no slight difficulty to make out correctly the individual elements of complicated structures, such as are shown by sections through the wood, we will therefore endeavour to obtain information by another method. We call to our assistance the so-called treatment by "maceration." For this purpose we place in a wide test-tube some pieces of chlorate of potash, and pour over them enough nitric acid to completely cover the pieces *; then lay in this fluid longitudinal sections, not cut too thin, of the tissue to be investigated, and warm it over a flame until active evolution of gas ensues. We allow the reagent to work for some minutes, and then pour the whole into a large evaporating dish, or saucer, full of water. The floating sections should be removed from this, by means of a glass rod, into another vessel filled with water, and from thence into a drop of water on an object slide. The maceration should not be carried on in the same room in which the microscopes are, as the vapours evolved are injurious to them. The preparation placed on the object slide can be torn to pieces with needles, and the individual elements thus separated. If the reagents have worked rightly, the **middle lamellæ** between the cells will be dissolved; the separation of the cells is therefore easy to complete. All the elements, which previously had to be studied in combination, will now be found

* If the proportions of these reagents are not right, red fumes of nitrous acid gas will be given off, and the tissue may be entirely dissolved. W. R. McNab has suggested a fluid composed of two drachms of nitric acid (of sp. gr. 1·10), and 3 grains of chlorate of potash, in which the tissue should be kept for a fortnight, when the constituent cells can be isolated. (*Trans. Edin. Bot. Soc.* vol. xi. p. 283.) [Ed.]

isolated under the microscope. They are usually well preserved, excepting that the lignin, by which the cellulose in the wood had been chemically altered, has been more or less completely removed, so that for the most part they stain violet with chlorzinc iodine. First we notice the **pitted ducts**, usually separated into segments at the places which indicate the annular diaphragms. Especially numerous in the preparation are the isolated **tracheïdes**; they are elongated, have tapering, rounded ends, and bordered pits. These pits, owing to the walls being swollen by the reagent, now present the appearance of narrow, obliquely ascending clefts; still, by focussing, you can get successive optical sections, which will show that the clefts widen towards their base. Where several tracheïdes remain joined together, the pits show a cross, because their cleft-like apertures are in opposite directions in adjoining cells. Besides ducts and tracheïdes, we also find in our preparation cells of the **wood-parenchyma**, with thin walls and large shallow pits; they are also recognisable by their conglomerated granular cell-contents. The wood-parenchyma cells, as we can easily determine, for the most part remain adhering together in threads, which simulate the wood-fibres, but differ from them in that their cavity is divided by cross-walls into several short superposed segments.

NOTE TO CHAPTER IX.

[1] Compare Russow, *Bot. Centralbl.* Bd. XIII., p. 140.

CHAPTER X.

STRUCTURE OF THE CONIFEROUS STEM.

Material Wanted.

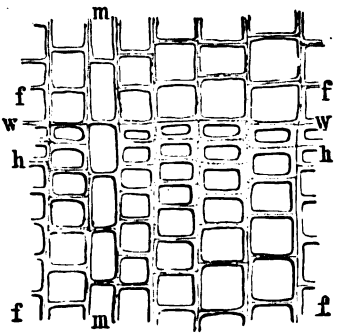
Pieces cut from the periphery of an old branchlet of the Scotch Fir (*Pinus sylvestris*). Cut, and placed in alcohol, in June or July. Placed in half-and-half glycerine and alcohol twenty-four hours before wanted for section-cutting. Likewise fresh.

We will first take up again the Scotch fir (*Pinus sylvestris*), already once investigated by us, and undertake a careful study of the structure of the stem. Since we have learned the method of growth in thickness of *Aristolochia*, we can do this with a much clearer comprehension.

It is characteristic of the Coniferæ that the entire secondary growth of the wood consists of but one kind of element, the **tracheïdes**, or, as here in the Scotch fir, of tracheïdes and individual strings of secondary wood-parenchyma. If you wish to find vessels in the Coniferæ, you must look for them in the medullary sheath, in the primary wood of the fibro-vasal bundle. Even in stems of half an inch in thickness this can be done very easily. In cross-sections passing through the pith, which is readily marked out to the naked eye by its darker colour, it can be seen that the inner margin of the ligneous mass consists of portions projecting into the pith and composed of elements with narrow cavities, and with somewhat brownish walls. In delicate radial longitudinal sections passing through the same region, we can determine that these elements are spiral vessels. Some such vessels, which possess at the same time spiral bands and bordered pits, serve as a transition to the tracheïdes having only bordered pits.

We will now institute a careful investigation into the region of the cambium; and we select alcohol-material as best for the purpose, since in fresh stems of the Scotch fir the cambium is usually torn through in cutting, while dry portions of stems do

not easily give good sections. The alcohol-material should be laid for about twenty-four hours in a mixture of equal parts of alcohol and glycerine, after which it can be prepared specially well. Alcohol-material offers this further advantage, in that the cell-contents are fixed. We select for the investigation pieces from the periphery of a tolerably thick branchlet, because the tracheïdes in the later-developed annual rings are larger. The pieces of stem are best laid in alcohol in the month of June or July, *i.e.*, at a time when the cambium is in full activity, and I assume that such a piece of stem is used for investigation. We examine the section in glycerine; in case, on the other hand, we wish to treat it with reagents, we first wash it in water. We begin with a delicate cross-section from the periphery of the stem—a section which extends through the bark, the cambium, and several annual rings of the wood. We first figure to ourselves on this section the things already known from our observations on bordered pits. We see the **tracheïdes** arranged in radial rows; from time to time such a row doubles in its outward direction. In outline, the tracheïdes are quadrangular, or else with five or six sides. In the wood formed in autumn the tracheïdes are narrower, and have thicker walls. After these thick-walled narrow elements succeeds passing outwardly without transition, the wood of the following spring growth, with its elements less strongly thickened and with broad cavities. This sudden change makes the year's limits visible to the naked eye [see Fig. 46*]. Parallel to the radial rows of the tracheïdes run the narrow, usually unilamellar (rarely more), **medullary rays**, generally distinguishable also through the starch-contents of their cells. In the *radial* walls of the tracheïdes [*i.e.*, those walls directed towards the medullary rays] are the bordered pits, the structure of which we already know. On the tangential walls [*i.e.*, the walls directed towards the centre and periphery of the stem] they are exceedingly rare. Between the tracheïdes and the starch-containing cells of the medullary ray are present very



[Fig. 46*.—Diagrammatic section, showing junction of autumn and spring wood in *Pinus sylvestris*. *h*, the latest autumn-formed elements; *w*, the boundary; *f*, the earliest spring-formed elements; *m*, a medullary ray. × 250 (after Prantl).]

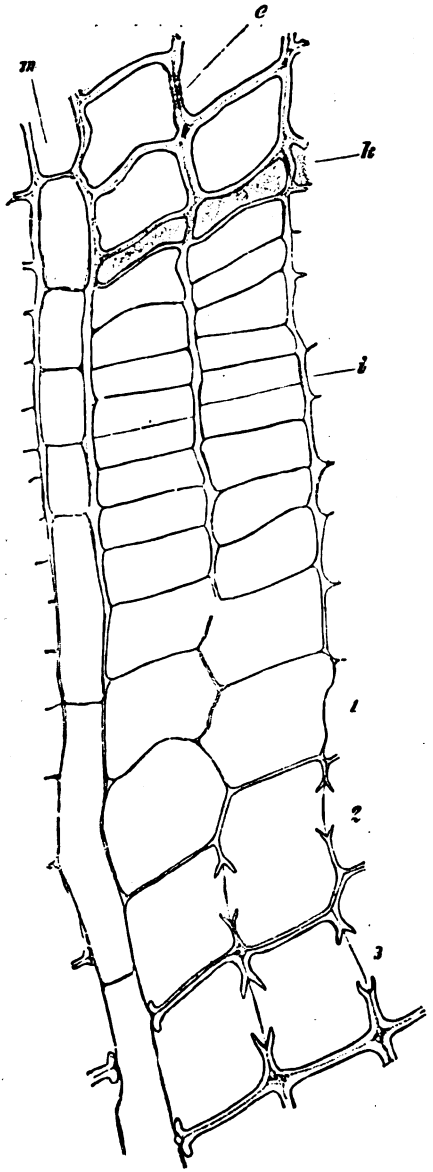


FIG. 47.—Part of a cross-section of a pretty old branch of *Pinus sylvestris*. The strip passes through the cambium (*c*, initial layer), and ends on the one side in the young wood, on the other side in the young bast. 1, 2, 3, stages in the development of the bordered pit; *m*, medullary ray; *e*, sieve-plate; *k*, flattened cells with brown contents, later on containing crystals (×540).

broad unilateral bordered pits, so broad that they almost represent the whole width of the wall of the tracheïde in contact with the ray-cell. By "unilateral" we must understand that only on the side of the tracheïde is the "border" of the pit developed. The closing membrane is usually bulged into the tracheïde; it appears to be without a "torus." The medullary ray cells, in those places with which the tangential walls of the tracheïdes adjoin, are provided each with projecting thickening ridge (compare with Fig. 47, the medullary ray *m* and the tracheïde walls impinging upon it). The section can, however, have been taken through a band of empty medullary ray cells, and these are then connected with the tracheïdes by pits which are bordered on both sides. In the immediate neighbourhood of the cambium we see (Fig. 47) the as yet incomplete tracheïdes, the so-called "young wood." Passing from the cambial zone, the walls of the cells increase rapidly

in thickness. In cross-sections, through older stems, we moreover often see the radial walls within the cambial zone itself to be thicker[1] (as in Fig. 47). What we must here call "cambium" consists in the **Initial layer** (*i*), assumed theoretically to be unilamellar, which by successive tangential divisions gives off mother-cells of the tissue on its wood and bast sides, and, from these, tissue-cells, still in course of division, which give rise to the elements of the wood and the bast. A sharp boundary cannot be drawn between the initial layer and the mother-cells of the tissue of the wood and bast respectively. The youngest partition wall in the cambium can be recognised, in that it passes without interruption to the radial lateral walls(*i*); somewhat older partition walls, on the contrary, join on to the side-walls at somewhat swollen points of origin. Towards the wood side the development of the bordered pits can be followed (1, 2, 3). The rows of tracheïdes are continued, through the cambium, into the rows of the elements of the bast, which maintain an equally strong radial arrangement. The cell-walls on the bast side become rapidly thickened; they have, however, a more dead-white, less glistening aspect than in the wood. On the radial walls of the broader bast elements, especially on the places where, in the wood, the bordered pits stand, are situated the sieve-areas (*e*); in very thin sections we can recognise the fine pores which pierce these areas.* Narrow, prominent, unilamellar bands of flattened cells alternate with the broad layers of sieve-tubes. These bands represent the bast-parenchyma. The greater number of the cells in the bast parenchyma are distinguished by a strongly refractive brown cell-content (*k*). In places somewhat further removed from the cambium, one or two crystals can be seen in the brown contents of these cells [**crystallogenous cells**]. As in the Scotch fir but one band of bast-parenchyma is formed yearly, the number of them can be used in estimating the age of any particular portion of bast. Between the cells containing crystals are others full of starch. Crystal or starch-containing cells, singly or in groups, are scattered also between the sieve-tubes. The medullary rays (*m*) are continued from the wood through the cambium into the bast, and in this latter their cells also in part contain starch. Only a comparatively narrow zone of the bast consists of turgid elements retaining their original arrangement. Beyond this zone the radial rows of cells are curved, the cell-walls begin to grow brown, the cavities of the cells become more or less con-

* Companion cells to the sieve tubes are not developed in the Gymnosperms.

tracted by pressure, so that the radial walls of the cells appear bent in waves or folds. Only the starch-containing cells of the bast or of the medullary rays enlarge notably; they become rounded, and appear now as more or less globular elements, thickly filled with starch. Finally, the sieve-tubes and cells containing crystals are completely crushed, and elongated tangentially, and now separate, like laminated membranes, the large starch-containing cells. The outer cortex appears now to consist of these last only. Further outwards in this bark we encounter narrow laminæ of cork, and outside these deep-brown dead tissue, cut off by them from the periphery.

Hitherto unmentioned are the strings of wood-parenchyma, which are shown in every cross-section of the wood, and which always contain a **resin-canal** (Fig. 48). In alcohol-preparations this canal has lost its resin contents. The cross-section through the wood cuts the resin-canals likewise in cross-section. Each of these presents itself as an intercellular passage (*i*), surrounded by a layer of large thin-walled cells (**Epithelial cells**, *e*). The walls of these cells have become brown; they contain a large nucleus and a peripheral layer of protoplasm. To these cells follow a second layer of cells, of similar form, but poorer in contents, and flattened; then a more or less complete sheath, here and there doubled, of large starch-containing wood-parenchyma cells. These last are surrounded by tracheïdes, or else impinge upon a medullary ray. As the history of their development shows, the resin canals arise *schizogenously*, *i.e.*, by the separation of cells at first in contact.

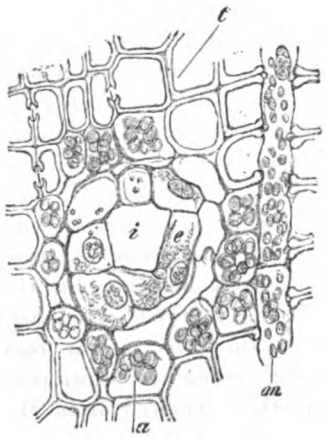


FIG. 48.—Resin-canal from the wood of *Pinus sylvestris*. *i*, the canal filled with resin; *e*, the epithelial cells surrounding the passage (canal); *a*, cells containing starch; *t*, tracheïdes; *m*, cells of a medullary ray (× 240).

For the purpose of comparison, let us now take a cross-section through a piece of fresh pine-wood, and determine that the resin canals are filled with **Resin.** This appears in the preparations in the form of strongly-refractive, extensible drops, often with irregular outline. If we run in a little alcohol, the resin-drops

quickly disappear. We can, moreover, stain them in characteristic fashion with the red colouring material of alcanna [2] roots [roots of *Alcanna tinctoria*], which we have already used to colour oils. For this purpose we take a cross-section through the wood of the Scotch fir, and lay it on the object-slide in a drop of water. We then take a similar thin section from the bark of a dried alcanna root, blow off from it the adhering particles, lay it upon the fir section, and cover with a cover-glass. Then run in a drop of about 50% alcohol under the cover-glass, and allow the object to stand for from half an hour to an hour. If now the alcanna bark is removed and the section of fir examined, the parts containing resin appear stained a beautiful dark red colour, while the other parts of the preparation remain entirely uncoloured.

Cross-sections through the alcohol-material treated with chlorzinc iodine show the walls of the tracheïdes yellow-brown; their innermost thickening layer, which is in contact with the limiting membrane [*i.e.*, the bright refractive line immediately surrounding the cell-cavity], is, however, partially stained violet. In the neighbourhood of the cambium, in tracheïdes which are not yet fully formed, the protoplasmic contents and nucleus are now easy to see. It can be settled equally definitely that the tracheïdes, when fully formed, lose all living contents. The cambium, with the youngest adjoining cells, has stained bright violet, the walls of the older portions of the bast a dark violet colour. The contents of the crystallogenous cells remain brown, while those of the cells of the periderm now appear reddish-brown. The inner surface, especially thin-walled, of the cells surrounding the resin-canal usually stains a dirty violet. Careful examination shows, moreover, that the closing membrane of the unilaterally bordered pits has stained violet, while that of the bilaterally bordered pits remains uncoloured.[3]

If we bring to bear on the wood of the Coniferæ, on sections taken through the cambium, the reactions for **lignin** which we have already tested, we can readily prove the gradual extinction of the lignin reaction in the neighbourhood of the cambium. Corallin also must, by virtue of its properties already known to us, stain the lignified cells quite differently from those unlignified. We obtain, indeed, very beautiful and instructive preparations when the sections are laid for some time in soda-corallin and then examined in glycerine. The lignified membranes are stained a deep red; towards the cambium this red disappears, and passes over into a pale yellow. In the bast the cell-walls have a pale

reddish-yellow coloration; the sieve-plates are coloured deep red, especially where they are covered with a callus layer. As corallin also stains starch-grains rose-colour, these consequently stand out quite sharply in the outer parts of the bast.

Now prepare a radial longitudinal section, using for the purpose the alcohol-material. The radial longitudinal section shows us in the wood the elongated tracheïdes with bordered pits, bluntly tapering at both ends, and by these ends interlapping with one another. The surface view of the bordered pit is already known to us. The bordered pits in the narrowest tracheïdes of autumn are small and few. Traversing the tracheïdes horizontally, we see the cells of the **medullary rays.** The medullary rays have usually little height [*i.e.*, in the direction of the length of the tracheïdes, and therefore of the length of the stem]; they can, however, occur up to sixteen cells high. They consist[4] of radially elongated cells, lying side by side in rows. The median cells contain starch, and show on the sides of the tracheïdes the large, shallow, unilateral, bordered pit; the upper and under rows of cells, from one to three in number, are empty, with small bordered pits, and show peculiar jagged (or serrate) projecting ridges on the walls directed tangentially. Similar rows of cells may also be interpolated in the median parts of very deep medullary rays. From their pits and want of living cell-contents these rows of cells resemble, in structure and relations, the tracheïdes of the wood, and on these grounds might be considered as tracheïdes; but we will confine this term to the elements of the ligneous portion of the fibro-vasal bundle alone. Here and there the radial longitudinal section may also cut through a string of secondary wood-parenchyma, and have laid bare its resin-canal. The parenchymatous cells surrounding the resin-canal are convex towards it, and just as broad as long; those more remote are clearly longer. In the larger medullary rays we can trace the course of a horizontal resin-canal, and can determine that ultimately the vertical and horizontal resin-canals intercommunicate. The **cambium** in longitudinal section shows as narrow elongated cells, with their end-walls more or less obliquely inclined, from which the elements of the wood and bast proceed, and as shorter, broader cells which are continued on both sides into the cells of the medullary rays.

In order to study the **sieve areas,**[5] we again take the alcohol-material, and lay the sections we have prepared in a watery solution of Aniline blue.[6] In this the sections need remain only

a few minutes, and then be transferred to glycerine. This latter permits the colour to remain only in the sieve-areas, removes it from all other parts of the section. The sieve-areas cannot now be overlooked in a microscopical examination. Their colour is beautifully blue and permanent, so that the preparations can be preserved. We can distinguish the sieve-areas even in the immediate neighbourhood of the cambium, and follow them into the parts in which the sieve-tubes have become crushed, and the sieve-areas have therefore lost their radial position. The sieve-areas, however, already lose their capability of coloration. The sieve-tubes have the form of cambium cells; they bear sieve-areas only on their radial walls, just as the bordered pits on the tracheïdes. The sieve-areas are, moreover, smaller than the bordered pits. They present the appearance of round or oval spots, which are collected into an indefinite number of somewhat angular, finely-punctate sections (Fig. 49). At some distance from the cambium the sieve-areas are covered by a homogeneous shining substance, staining sky-blue—the **callus-plate**. Further still these are again dissolved, the sieve-area is bare, and no longer colours at all. The sieve-tubes are here already functionless. It is not difficult to recognise that the active sieve-tubes contain protoplasmic contents, the nucleus, however, except occasionally, is wanting, already disappearing in the developing sieve-tube.

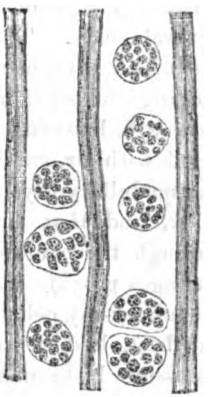


FIG. 49.—*Pinus sylvestris*. Parts of two sieve-tubes with sieve-areas (× 540).

The **crystallogenous cells** of the bast are distinguished in longitudinal section by their brown contents; they are comparatively short, end commonly with horizontal walls, and have apparently arisen from horizontal division of the cambium-cells. They contain numerous prismatic crystals lying over and by one another. The **starch-cells** likewise stand out clearly. They are still shorter than the crystallogenous cells, lie in rows one upon the other, and are also interpolated singly or in longer rows between the crystallogenous cells. Later these starch-containing cells swell very considerably.

The cells of the **medullary rays** can be readily followed from the wood into the bast; they retain, moreover, their essential structure, but lose their characteristic pits; the inner, starch-con-

taining rows are here also usually accompanied, both above and below, by cells free of starch. These last cells are narrower and longer than those which contain starch, quickly lose their living contents and collapse. All the elements of the medullary rays within the bast remain thin-walled. The horizontal resin-canals, also, in the large medullary rays, pass out of the wood into the bast.

It is desirable to take also a radial longitudinal section from fresh material. If this is examined in water, we can determine that even in the cambium and the neighbouring youngest elements, as well as in the parenchyma around the most recently developed resin passages, but specially, however, in the starch-containing cells of the medullary rays of the woody body and of the bast, an active streaming of the protoplasm exists. In the medullary rays of the wood this can be followed back through several years. The tracheïdes, and the tracheïdal cells of the medullary rays, have for the most part become filled with air in cutting the section. A careful examination of the medullary rays will, however, show that extremely fine intercellular spaces, filled with air, run in a radial direction between the full cells of the medullary rays and the neighbouring tracheïdes of the woody body, and that they also, following the medullary rays, pass through the cambium into the zone of bast. These intercellular passages provide for the necessary access of air to the living me-medullary-ray cells.—A longitudinal section which passes through the bast, laid in ammonium-chloride, molybdic ammonia, or in potassium bichromate, shows us that the crystallogenous cells contain tannin.—If now we remove by radial longitudinal sections up to about the twentieth year's ring from the cambium, we shall see that the cells of the medullary rays gradually lose their living contents, and in its place become filled with resin. The tracheïdes also, especially those of autumn, contain in many parts resin. The living elements have now disappeared from the woody body; we have dead heart wood (**duramen**), in contradistinction to the sapwood (or **alburnum**), traversed by living medullary rays.

The tangential longitudinal section, which we likewise prepare from the alcohol-material, must be taken from at least two places, viz., one in the wood and one in the bast, and in both cases in the immediate neighbourhood of the cambium. The section through the wood shows the tracheïdes, and the sections of the **medullary rays**, each cross-section of these tapering at its ends, and the

whole therefore having a spindle-like outline, since the cells at both edges (or ends) gradually become narrower, and the final cells are unilaterally pointed. The simplest medullary rays are in cross-section 3 cells high; most are about 8 cells, while with some the height can increase up to as many as 20 cells. The simplest are always but one cell broad; the highest can in their central part be several layers thick, and these latter have then a resin-canal, which is shown in section. The section may have also cut through a vertical resin-canal, which will present the same appearance as in the radial longitudinal section. A section of the bast which will answer our requirements cannot be obtained without trouble. There is no recourse but to prepare from an older portion of bast, beginning externally, a considerable number of successive sections, till we have reached the young wood. We examine these sections with a low power objective, and look for such as contain still active **sieve-tubes.** By this means we can obtain information about the callus plate, which at once attracts the eye, even without coloration and with slight magnification, as a strongly refractive pad attached to the cell-wall. The sections of the sieve-areas can be best studied in chlorzinc iodine, to which we have added an equal bulk of dilute watery potassium-iodide iodine solution. The figure of the sieve-area in this view is the same as in the cross-section, but the number of the cut sieve-areas is very great, and therefore it is more easy to obtain a favourable section. They will be found most quickly at the edges of the section. The sieve-areas (Fig. 50, *A*) are seen in profile, within the radial walls of the sieve-tube which we have cut through with the razor. The walls themselves are somewhat swollen in the chlorzinc iodine solution, and have acquired a violet coloration. The sieve area is, so far as it appertains to a still active sieve-tube, stained reddish-brown. This colour proceeds from the strings of plasma which penetrate from both sides into the thickness of the sieve-area. It appears

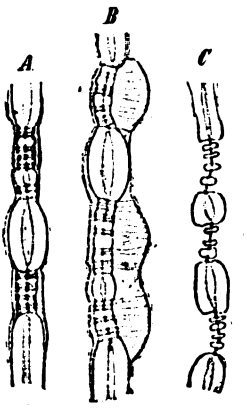


FIG. 50.—*Pinus sylvestris* Parts of the walls of sieve-tubes after treatment with chlorzinc iodine. *A*, before the formation of the callus plate; *B*, after its formation. *C*, from a sieve-tube which has passed its period of activity (× 540).

then as if the sieve-area were pierced by reddish-brown rods (compare the figure). The callus-plates (*B*) have coloured reddish-brown, provided the chlorzinc iodine solution was not too concentrated, and has not acted as a solvent. The sieve-areas of sieve-tubes which have lost their function (*C*) appear bright violet; the strings of plasma and the callus-plates have disappeared from these. If we stain such a tangential longitudinal section with aniline blue, and examine it in glycerine, the brilliant blue callus-plates at once attract attention. We can easily follow on the one side the growth, and on the other side the disappearance, of them.

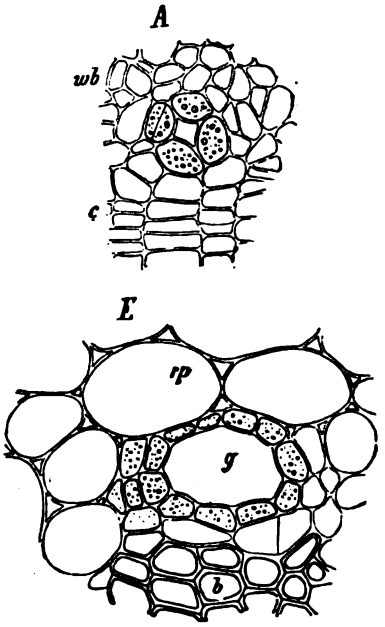


FIG. 50*.—Resin passages in the bast of a young stem of *Hedera helix* in transverse section (× 800). *A*, early stage; *E*, later stage; *g*, the resin passage surrounded by its secreting cells; *c*, the cambium layer; *wb*, soft bast; *b*, bast fibres; *rp*, cortical parenchyma. (After Sachs).

The resin-canals which we have here met with, and which in the cortical parenchyma of Conifers often attain considerable size, can have their origin well studied in the ivy (*Hedera helix*). Cross-sections of young stems will show the resin-canals in the central pith as well as outside the xylem ring, even in quite close contiguity to the cambium-ring. In the same section they will be met with in different stages of development, from a group of four cells, having between them a barely recognisable intercellular space, to fifteen or twenty cells surrounding a large cavity. The cells are at once recognisable by their granular contents. The mode of origin of the resin-passage is easily observable. It arises through the separation of the secreting cells, while these cells divide radially, and more rarely tangentially (Fig. 50* *A*), and thus surround a cavity continually enlarging up to a certain point. The resin passage is therefore **schizogenous** (compare Fig. 50*).

NOTES TO CHAPTER X.

[1] Sanio, *Jahrb. f. Wiss. Bot.* Bd. IX. p. 51. E. Strasburger, *Zellhäute*, p. 39. Kny, *Anat. d. Holzes von Pinus sylvestris*, Bot. Wandtafeln, VI. Abth.

[2] According to N. J. C. Müller, *Jahrb. f. wiss. Bot.* Bd. V. p. 398.

[3] Russow, *Bot. Centrabl.*, 1883. Bd. XIII. p. 140.

[4] For particulars see De Bary, *Comparative Anatomy of Phanerogams and Ferns* (Eng. trans.), p. 490.

[5] Janczewski, *Mém. de la soc. d. sc. nat. de Cherbourg*, vol. XXIII. p. 260; E. Strasburger, *Zellhäute*, p. 57; Russow, *Stzbr. Dorp. naturf. Gesellsch*, 17th February, 1882, p. 264.

[A French translation of the researches of Russow on sieve-tubes, and reprint of those of Janczewski, will be found in *Ann. des Sciences nat. Botanique*, 1882.)

[6] K. Wilhelm, *Beitrage zur Kenntniss des Siebröhren-apparates*, 1880, p. 36; Russow, *Stzber. d. Dorp. naturf. Gesellsch*, 1881, p. 63.

CHAPTER XI.

STRUCTURE OF THE STEM OF THE LIME; BICOLLATERAL VASCULAR BUNDLES OF THE CUCURBITACEÆ; SIEVE-TUBES.

Material Wanted.

Twigs of Lime (*Tilia europæa*), about ¼ inch thick.
Stems of a Gourd (*Cucurbita pepo*), about ¼ inch thick, cut about ⅓ yard from apex. Fresh, and in alcohol.

As our next object of investigation, we choose the Lime (*Tilia parvifolia*), or any form of the aggregate species, known under the name of *Tilia europæa*. A cross-section through a twig about ⅛ of an inch in thickness shows us a pith consisting of large cells, the air-containing cells of which are grouped, rosette-like, around individual narrower cells, filled with finely granular brown contents. In the outer part of the pith lie gum-reservoirs, forming hollows in the parenchymatous tissue, but which, however, are already empty. At its outermost limits the pith consists of smaller cells, the cells filled with finely granular contents. Into this small-celled tissue project the primary wood portions of the vascular bundles. The "unwinding" spiral vessels, from which the spiral thickening thread can be unrolled, are at once noticeable in the cross-section, from the thickening bands standing out here and there. We can count about 5 annual rings in the cross-section of a twig of ¼ inch thickness, and we shall perhaps notice that the successive annual rings can be of very varying thickness. If now we examine more closely the individual annual rings, we shall observe, first of all, the great vessels (Fig. 50A), which are especially produced in the spring, and mark at once the limits of the year. Further in the year's growth the broad ducts either arise singly or in isolated groups; in the last phase of the year's vegetation the cambium produces only elements with narrow cavity. If we run in to our preparation a little iodine solution, besides the medullary rays a number

of other cells will be noticeable for their plasmic contents, and commonly also for their starch. They are scattered amongst the other elements of the wood, but commonly are connected together laterally, and with the medullary rays also, into tangential bands. These cells are the wood-parenchyma (*p*). The tracheïdes (*t*), somewhat resembling the vessels, and connected with them by transitions, are recognisable by the absence of plasmic contents, and by the bordered pits with which they impinge on similar elements or on the vessels. Lastly, we recognise the wood-fibres (*l*),

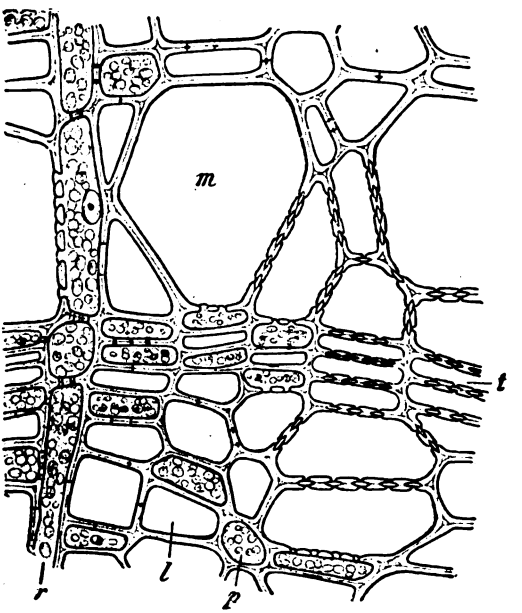


FIG 50A.—Cross-section through the wood of *Tilia parvifolia* (alcohol material). *m*, a wide pitted duct; *t*, tracheïdes; *l*, wood-fibres; *p*, wood-parenchyma; *r*, medullary ray. (× 540.)

likewise poor in contents, usually containing air, and provided with very fine pits. These wood-fibres form the greater part of the wood. The narrow-cavitied autumnal wood consists only of wood-parenchyma, tracheïdes (*t*), and wood-fibres; and it is bounded by the spring wood, consisting especially of vessels and tracheïdes (cf. Figure). Very delicate cross-sections show that practically no pits traverse the walls of the vessels and tracheïdes on the sides towards wood-fibres, and that on the sides towards

the wood-parenchyma cells, on the other hand, they are only unilaterally bordered. The border is only developed on the side of the vessel or tracheïde, and the closing membrane (which shows no torus) bulges out into it (*vide* Figure, below *m*). The fine pits which communicate between the wood-fibres are slightly bordered at their base, as also are the isolated pits which here and there traverse the wall of a wood-fibre in the direction of a tracheïde or of a vessel. Between tracheïde and wood-parenchyma these pits appear only unilaterally bordered, but owing to their slight width this can scarcely be determined with certainty. The cells of the medullary rays have the same pit arrangements as the wood-parenchyma, but owing to their radial elongation the

FIG. 50B.—Cross-section through the bast of *Tilia parvifolia* (alcohol material). *v*, sieve-tubes, at *v+* exposing sieve-plate; *c*, companion-cell; *p*, bast-parenchyma; *k*, crystal-logenous cell; *l*, bast-fibre; *r*, medullary ray. (× 540)

medullary ray-cells are sharply contrasted with the surrounding elements. In the outer limits of the woody body the cambium ring is readily recognisable by its flat, thin-walled, radially-arranged elements. On the other side of the cambium the keel-shaped tapering portions of the bast are at once noticeable [Fig. 50**]. In these an alternation of tangentially-arranged white and dark layers is shown. The glistening white layers are composed of numerous closely-combined bast-fibres, the walls of

which are thickened almost to the disappearance of the cavity. The cavity of each individual cell shows only as a black point. The layers (Fig. 50B) are of unequal thickness, and are usually hollowed, furrow-like, on their outer side. The darker layers between the white consist, passing from the outside inwards, of a layer of somewhat broader crystallogenous cells (*k*), then of about two layers of starch-containing bast-parenchyma cells (*p*), and after these of many sieve-tubes with broad cavities (*v*). From the corners of these sieve-tubes the narrow companion-cells (*c*) are cut off in a very conspicuous fashion. A layer of very narrow bast-parenchyma cells (*p*) serves to separate the sieve-tubes and companion-cells from the next succeeding layer of bast-fibres (*l*). It is necessary that these observations should be made at a short distance from the cambium, because further, from about the eighth layer of bast-fibres onwards, the sieve-tubes have passed their active period and contain air. The cross-section usually cuts through some of the strongly inclined sieve-plates, these being readily recognisable from their sieve-pores, and contents aggregated on both sides (v^+). Probably twice as many layers of secondary bast-fibres can be counted as there are annual rings in the wood. Apart from the first two years, two layers of bast-fibres are developed pretty regularly in each year. The outermost edge of the bast is occupied by the strings of primary sclerenchyma, which in no way differ from the secondary bast strings. The primary medullary rays in the ligneous body are usually two (here and there, however, more) cells thick; the secondary medullary rays only one cell thick. They can be followed through the cambium into the primary cortex, or into the bast respectively. The outer ends of the primary medullary rays are considerably broadened into a funnel shape, and separate the portions of bast. They segment the bast into the form of inverted radiating keels. The numerous tangential divisions in these ends of the medullary rays have caused an arrangement of the cells into tangential rows. The outer end of the medullary rays, and the primary portions of the bast, plunge into the actively-living green primary cortex. In the outer parts of the medullary rays, and in the primary cortex, are numerous crystallogenous cells. Further outwards, the cells containing chlorophyll can be easily recognised by their white walls, especially strongly thickened in the corners, as collenchyma-cells. The surface of the stem is covered by a regularly developed periderm, whose flat

cells are of a stronger and stronger brown colour, according to their age, *i.e.*, passing from inside [the youngest] outwards. A general idea of the arrangement of the parts can be obtained from the accompanying diagrammatic view of the cross-section of a four-year old stem of the lime, the description of which is given at the foot of the figure (Fig. 50 **).

In radial longitudinal sections, we can make out that the ducts of the secondary wood have bordered pits, and that moreover between the pits they have spiral bands, as an innermost thickening layer. The ends of the ducts joining on to one another, show an oblique wall, perforated with a single large opening. Besides the vessels, and always connected with them by intermediate forms, can be seen tracheïdes, especially in the autumn wood, thickened in the same way as the ducts, but tapering at both ends, and closed. Between the ducts and the tracheïdes lie elongated "wood fibres" (libriform fibres), tapering at both ends, having small scattered pits, slightly enlarged at their base (bordered), and narrow wood-parenchyma cells, filled with oil-drops or with starch, simply pitted (not bordered), ending with horizontal and likewise pitted walls. The wood-fibres are longer than the tracheïdes, like these without living contents, and containing only water; functionally, at all events, nearly akin to them. The pits of the wood-fibres open into the cell-cavity by a narrow cleft, which in cell-walls in contact with one another are inclined in opposite directions; therefore, with intermediate focussing, they show a small cross in the pit. In these wood-fibres, as almost universally in the mechanical elements (stereïdes), the cleft-like pits mount towards the left, *i.e.*, they follow a left-handed spiral line.[1] In the wall of the ducts the pits are large and numerously developed only where one vessel impinges on another, or on a tracheïde.

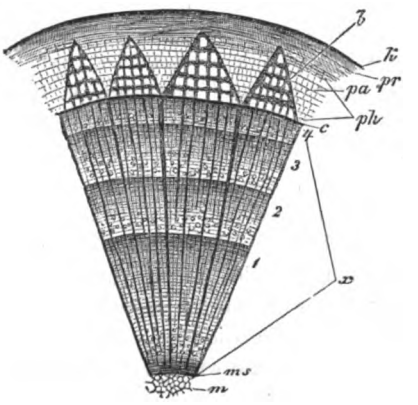


FIG. 50**.—Diagram of part of a cross-section of a twig of the Lime, 4 years old (slightly magnified): *m*, pith; *ms*, medullary sheath; *x*, annual rings of wood 1, 2, 3, 4; *c*, cambium; *ph*, bast; *pa*, primary medullary rays; *b*, bast-fibres; *pr*, primary cortex; *k*, cork. (After Prantl).

Those parts of their walls impinging on the wood fibres are just as sparsely pitted, and the pits as small, as these. Where the wood-parenchyma cells impinge on a duct, a corresponding influence on the pits can be likewise noted; the pits of the duct are bordered only unilaterally, on the side of the duct. The fibres of the autumn wood are particularly narrow. The medullary rays pass through the wood, as horizontal bands of considerable height; they consist of rectangular, radially elongated cells, which contain starch, and have very numerous pits, especially on the tangentially-placed walls. In the bast we can see the very long, strongly-thickened, white bast-fibres, pointed at both ends; between the strings of bast-fibres short parenchymatous cells, with horizontal end-walls, and containing starch, and here and there also prismatic crystals; and the sieve-tubes, whose sieve-plates, if placed obliquely, are divided by horizontal bars into several sections. Besides these, the collenchyma and cork offer points of interest. As, however, the collenchyma and cork-cells are just as high as broad, the figure in the longitudinal section completely resembles that in the cross-section.

The tangential longitudinal section confirms the conclusion as to the considerable height of individual medullary rays derived from the radial longitudinal sections. The medullary rays are either one cell thick in their entire height, or double in the middle. For the rest, we find the elements again as in the radial section.

The tangential tapering of the tracheïdes and wood-fibres can now be well seen, and it is easy also to make out that the wood-parenchyma cells are connected together into threads which simulate in form the wood-fibres. Each of these threads has been derived from the segmentation of a single cambial cell, and the tangential tapering of the various elements in general has arisen from the like form of the cambial cells.

Chlorzinc iodine stains the ligneous part yellowish-brown, the cambium violet. In the bast is shown a beautiful alternation between the violet thin-walled zones and the bright yellow, thick-walled bast fibres. The elongated medullary rays of the primary cortex are violet, the cork is reddish-brown.

Corallin stains the wood cherry-red, the bast-fibres quite a strikingly beautiful bright rose-red. The sieve-plates stand out clearly even in the cross-section by their fox-red coloration.

For careful study older stems should be chosen, and alcohol

material is to be preferred to fresh stems, as in the latter the air abundantly contained in numerous elements disturbs observation.

On account of the difficulty in studying the structure of the secondary wood, we will here bring the "maceration"

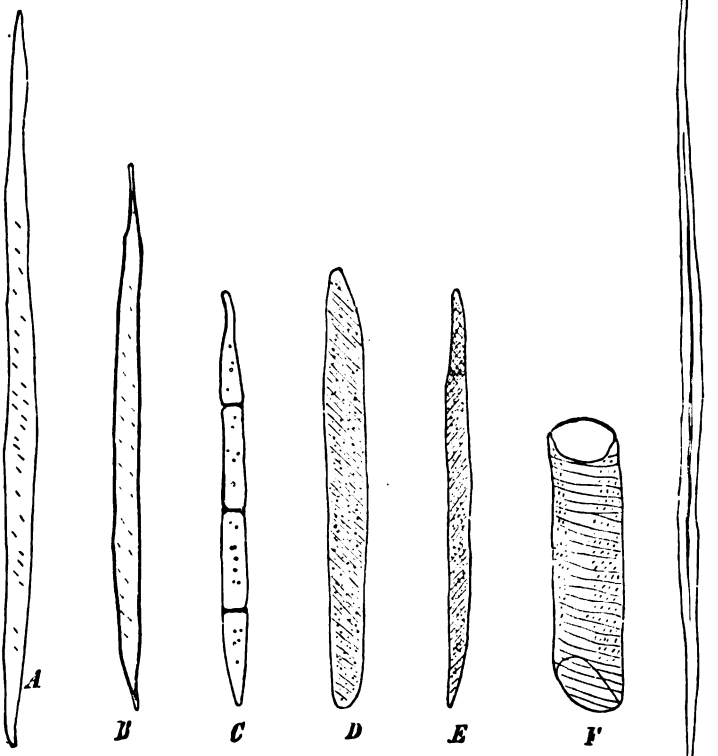


FIG. 51.—*Tilia parvifolia*. Elements of the secondary wood and bast, isolated by maceration. *A* and *B*, wood-fibres (libriform); *C*, wood parenchyma; *D* and *E*, tracheïdes; *F*, segment of a duct; *G*, bast fibre (× 180).

method again to our aid, and examine the elements isolated from one another. We proceed exactly as with *Aristolochia* (p. 112 *et seq.*), and separate the macerated sections with the needles. The preparation will then show the especial preponderance of the wood-fibres (Fig. 51, *A*, *B*). The swelling of the walls causes the pits to appear still smaller; they appear as oblique clefts. The short parenchymatous cells, recognisable by their contents, lie

scattered between the wood fibres, and are either separated or, usually, still united into threads which resemble in outline the wood-fibres (*C*). We find, further, but in smaller number, the spirally-thickened tracheïdes, in outer contour either more resembling the wood-fibres (*E*), or more approaching the ducts (*D*); finally the ducts themselves, either separated into segments (*F*) or else forming long-tubes. In the preparation the very long and narrow cavitied bast-fibres (*G*) will also strike us. Careful examination of the tracheïdes and vessels enables us to determine that the cleft-like apertures of the pits show an opposite direction to the spiral band, and in the broader ducts the slope is much steeper than that of the spiral band, while in the narrower tracheïdes there is about equal steepness. The tracheïdes, as we have already seen, can be very like to the ducts; in fact, a sharp delimitation between the broadest tracheïdes and the narrowest ducts can here scarcely be made. In our descriptions here and in other places we have been guided by the outer form, and have attributed the tubular forms to vessels, and the fibre forms to tracheïdes.

In almost all the Cucurbitaceæ, from amongst which we select *Cucurbita Pepo* (the pumpkin) for investigation, the vascular bundles have two bast portions, one on the outer, the other on the inner side of the wood. These vascular bundles are therefore constructed **bicollaterally**. The outer bast is separated from the wood by the cambium, the inner bast impinges immediately on the inner bounds of the wood. In order to see the vascular bundle fully formed, stems of at least $\frac{1}{3}$ inch thick should be examined, and in parts which are distant about half a yard from the growing point. In stems of $\frac{1}{8}$ to $\frac{1}{4}$ inch in thickness, or nearer the growing point, the larger ducts are not yet complete. We take for first examination alcohol-material, for this offers various advantages. The vascular bundle has no sheath, and is not sharply delimited from the surrounding tissue. Better-defined figures can be, however, obtained if the section is submitted for a short time to the action of aniline blue, and afterwards examined in glycerine.

The parts appertaining to the fibro-vasal bundles appear more darkly stained than the ground tissue. If we suppose the inner bast removed, the figure approaches so nearly to the dicotyledonous fibro-vasal bundles already known to us as those of *Ranunculus* and *Chelidonium*, that we should have no difficulty in placing it amongst them. We examine first a cross-section of a fully-developed fibro-vasal bundle, with perfect ducts, always looking for the most normal cases where two large ducts are present. These ducts are amongst the broadest that are known anywhere. Between them lie the cells of the primary parenchyma, with tolerably broad cavities, usually elongated somewhat radially, and with their walls thickened as strongly as the vessels, and always clearly reticulately. Succeeding these internally are vessels whose diameter falls considerably short of that of the two great ducts, and in passing inwards is still further reduced. Between these vessels lies the thin-walled primary wood-parenchyma, which is continued beyond the limits of the innermost vessels. Upon this thin-walled tissue finally impinges the inner bast, consisting of broad-cavitied sieve-tubes, of narrower companion-cells, and of somewhat broader bast-parenchyma cells.

We have here an easy opportunity of observing the sieve-plates, which here are disposed horizontally, in surface view (Fig. 52, *A*). The companion-cells (*s*) stand out particularly sharply with their dark blue-stained contents. At the outer side of the wood can be seen the thin-walled radially-arranged cells of the cambium layer, following immediately after the two largest ducts, and the thick-walled cells of the wood-parenchyma lying between them. Then follows the outer bast, constructed just as in the inner. In both portions of the bast the sieve-plates, where such are cut, are readily recognisable from their areolation. Each areole appears, according to the stage in its development, to be pierced by a large or small pore. In older sieve-tubes the pores are narrower, and lined with a highly refractive substance (as in Fig. 52, *A*). The sieve-plate also often appears to be covered with a mass of substance stained violet-blue. In the narrower sieve-tubes, at the outer and inner margin of the fibro-vasal bundle, the section has also probably laid bare a callus-plate, which stands out brightly as a homogeneous mass, coloured a beautiful sky-blue. If we focus more deeply into such a callus-plate, we can recognise in it the network of the sieve-plate. The fibro-vasal bundles, as examination of the cross-section with a low power shows, are arranged in

two concentric rings, about five in each ring. The fibro-vasal bundles in the outer ring are inside the angles of the stem, those of the inner ring alternate with the outer ones. The protection of the inner parts of the stem is served by a ring of sclerenchyma-fibres, the elements of which have stained far more deeply than the large-celled ground tissue. Externally to this ring is a cortical parenchyma, containing chlorophyll, and then a typically developed, stellately interrupted shining white collenchyma. At the interruptions in the collenchyma, the cortical parenchyma extends to the epidermis, which has its stomata at these places. In the interior the stem is hollow. Cross-sections through thinner

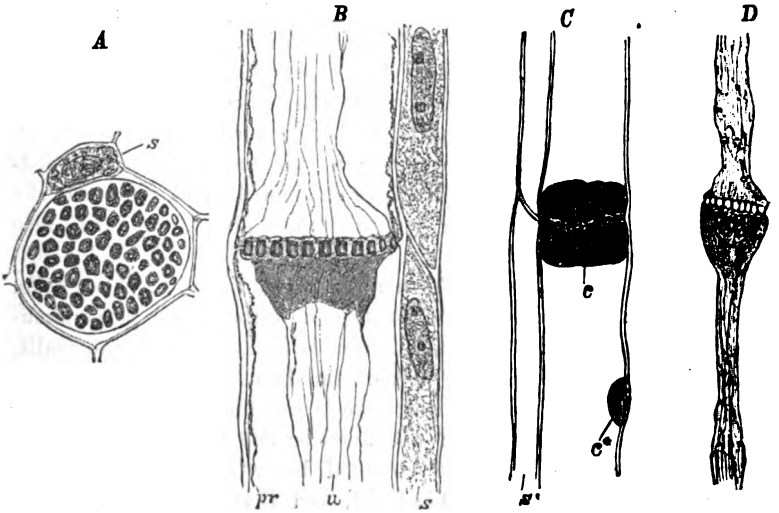


FIG. 52.—*Cucurbita Pepo*. Parts of sieve-tubes. *A*, in cross-section, *B* to *D*, in longitudinal section. *A*, a sieve-plate seen from above. *B* and *C*, side-view of the adjoining parts of two sieve-tubes. *D*, the connected parts of the slime-strings of two-sieve tubes, after the action of sulphuric acid. *s*, Companion-cells; *u*, string of slime; *pr*, protoplasmic sac; *c*, callus plate; c^*, small unilateral callus-plate from a lateral sieve-area (× 540).

stems, from $\frac{1}{4}$ to $\frac{1}{2}$ inch thick, show the largest ducts, and the elements lying between them, still in course of formation. It not infrequently occurs that of the two great ducts only one is completely formed, while the other, on the contrary, is obliterated; the former then attains an enormous diameter. In many cases both ducts can be obliterated. Finally isolated cases are met with where both ducts are present, and both are as great as if only one had been produced.

Radial longitudinal sections, which have cut through a fibro-

vasal bundle correctly, show us that the narrowest vessels are annular and spiral vessels; the broader are pitted, with annular horizontally-situated diaphragms. The two greatest ducts have irregular, reticulately thickened walls, and in the meshes of the net numerous pits. Longitudinal sections will be not infrequently obtained, which show the largest ducts provided with a complete cross-wall. Then there are still present in the cells a nucleus and a thin peripheral layer of protoplasm. Many cross-walls will show, however, to be already strongly swollen in their middle part, and therefore in optical section show as biconvex lenses. Longitudinal sections of the next older parts of the stem show us these septa, or partition walls, finally reduced in places to a narrow ring, attached to the side wall of the duct. The protoplasmic contents of the cells, as well as the nucleus, have then disappeared. The thin-walled tissue between the narrower vessels consists of elongated parenchymatous cells, ending on one another with horizontal walls, and therefore being a thin-walled primary wood-parenchyma. The more strongly-thickened cells between the great ducts are plentifully but shallowly pitted, and have also pitted cross-walls, and belong, therefore, to the thick-walled primary wood-parenchyma. As a special peculiarity of these cells, we notice the undulating course of their walls placed perpendicularly to the ducts. This undulation is occasioned by the walls, in joining on, avoiding the pits of the duct. In these wood parenchyma cells is found protoplasmic sac and nucleus. Where pitted ducts impinge upon one another the pits are bilaterally bordered; where they are bounded by wood-parenchyma the pits are only unilaterally bordered, and that on the side of the fibro-vasal bundle.

On both sides of the fibro-vasal bundle we can, by means of longitudinal sections, conveniently study these so unusually large sieve-tubes[2] (Fig. 52, *B*). For this purpose we lay the longitudinal section for a short time in aniline blue, and afterwards examine it in glycerine. After lying a pretty long time in this latter, the cell-walls are more or less completely decolorized, while the contents of the sieve-tubes retain the colour. Almost all the sieve-plates are placed horizontally; only a few have an inclined position. Most of them appear covered with a highly refractive callous substance, and show, corresponding to this, a not inconsiderable thickness (Fig. 52, *C*). From this peculiarity they are noticeable with but low magnification. In our aniline-blue

preparations these sieve-plates are coloured clear blue. In the interior of the sieve-tubes in question, which show these sieve-plates, is visible a contracted, sac-like, axial string (*u*). This is a string of protoplasmic slime or mucus, which broadens at its ends, and almost completely covers the sieve-plate. It is stained indigo-blue. The ends adjoining the sieve-plates are usually more densely full of contents, and form a terminal "mucous plug." This accumulation of contents can be observed either on both or only at the upper end of the sieve-tube. Besides the axial sac, the sieve-tube shows, by very careful examination, a thin peripheral layer of protoplasm; the peripheral layer may be extremely thin, and adhering everywhere closely to the wall of the sieve-tube. A nucleus is not present. In somewhat younger sieve-tubes the slime-string can be often seen to push out through the pores of the sieve-plate from one [constituent cell of the] sieve-tube towards the other in the form of bladder-like or worm-like prolongations. In older sieve-plates such prolongations can no longer be seen; the callous substance has augmented and contracted the sieve-pores. Through these contracted pores the slimy contents of one constituent cell of a sieve-tube are continuous with those of another (as in *B*). The sieve-plates with their callous coverings (Fig. 52, *C*), are noticeable, as previously in the cross-section, on both inner and outer side of the fibro-vasal bundle. These plates of callus are clearly indicated by their higher refraction, and are stained sky-blue. In the middle of the callus-plate the sieve-plate is more or less clearly recognisable. The callus-plate consists, therefore, of two halves, belonging to adjoining cells of the sieve-tube, and are united together through the pores of the sieve-plate. A delicate perpendicular striation is often to be recognised in the callus-plate (compare Fig. *C*), and these striæ always pierce the pores of the sieve-plate. Where two sieve-tubes are in contact laterally, small sieve-areas are formed on the common side-walls. These also later on possess a unilateral (*c**) or bilateral callus-plate, and are thus very evident. By the side of the sieve-tubes, and clearly corresponding in length with the constituent cells of these, run the companion-cells (*s*). They have abundant protoplasmic contents, and a nucleus. Between sieve-tubes and companion-cells can be seen numerous horizontally elongated and corresponding pits. The sieve-tubes in course of development show in their peripheral protoplasmic layer drops of mucus stained indigo-blue. These drops

of mucus dissolve in order to form the mucus-string. It is very instructive to treat a longitudinal section of the alcohol-material with concentrated sulphuric acid. The walls of the sieve-tubes and the sieve-plates are dissolved. The mucus masses, however, remained unaffected, and we can thus obtain preparations of sieve-tubes, of which the contents are dissolved, and which show as in *D* in Fig. 52. Such preparations demonstrate in the clearest manner the union which exists between the contents of sieve-tubes which are in contact with one another. These preparations can be washed by running water under one edge of the cover-glass, and withdrawing it from the opposite edge by means of blotting-paper, and can then be stained with a drop of aniline-blue.

In order to obtain a thoroughly correct idea as to the normal distribution of the contents of the sieve-tubes, it is necessary to have recourse to still another method of preparation. It is worth while to fix the contents of the sieve-tubes with boiling water, without, however, having previously injured the plant, because dislocation of the contents of the sieve-tube would be induced thereby. Uninjured shoots, while still in union with the mother plant, are killed by being immersed for about five minutes in boiling water.[3] Material thus fixed can be either investigated direct, or can be preserved at will, and without further change, in alcohol.

For comparison, it is necessary to prepare some longitudinal sections of fresh material. In these also the sieve-plates show just as clearly as in the alcohol-material. The accumulations of slime at the sieve-plates can be well seen; but the slime nowhere shows as a special string withdrawn from the side-walls of the sieve-tube. This appearance therefore arises from the action of the alcohol.

NOTES TO CHAPTER XI.

[1] Compare Schwendener, *Das mech. Princip*, p. 8.

[2] Compare herewith especially De Bary, *Comparative Anatomy of Phanerogams and Ferns* (Eng. trans.), p. 172; K. Wilhelm, *Beiträge zur Kenntnis des Siebröhren-Apparates dicotyler Pflanzen;* E. v. Janczewski, *Etudes comparées sur les tubes cribreux, Mém. de la Soc. nat. des sc. nat. de Cherbourg*, T. XXIII. (reprinted in *Annales des sc. nat. Bot.*, 1882); Russow, *Sitzbr. der Dorp. naturf. Gesellsch.*, Jahrg, 1881 and 1882 (the last two also in *Annales des sc. nat. Bot.*, 1882).

[3] Compare Alfred Fisher, *Ber. d. Deut. bot., Gesell*, 1885, p. 230.

CHAPTER XII.

AXIAL FIBRO-VASAL CYLINDER AND SECONDARY INCREASE IN THICKNESS OF ROOTS.

Material Wanted.

Root of the Onion (*Allium Cepa*). Fresh (or in alcohol).
Root of the sweet Flag (*Acorus calamus*). Fresh (or in alcohol).
Root of *Iris florentina*. Fresh (or in alcohol).
Adventitious root from the runner of *Ranunculus repens*. Fresh (or in alcohol).
Roots of the Yew (*Taxus baccata*), from $\frac{1}{25}$ inch thick up to $\frac{1}{12}$ inch thick. Fresh.

With the structure of the **axial fibro-vasal cylinder** of roots [1] we will first make ourselves acquainted upon the root of *Allium Cepa* (the garden onion). Abundant material for investigation can be at any time secured by allowing the onion to grow in water in a hyacinth glass. Figure 53 shows us a cross-section from the base of a strong **adventitious root** thus obtained. The epidermis and the very thick cortical tissue are left out of the drawing, but of the latter we see the cells which bound the axial cylinder as a "**bundle-sheath**," or **endodermis** (*c*). The endodermis, or bundle-sheath, shows a characteristic dark shading upon the radial walls of its component cells. This shading is produced by the wavy flexure of the median portion of the walls. Such an endodermis is always unilamellar.[a] The centre of the fibro-vasal cylinder is occupied in this instance by two broad **scalariform vessels** (*sc*); in other cases, however, either only one, or more than two, can be found. If the root is not old enough, the central and perhaps also the adjoining vessels are thin-walled and not fully developed. Adjoining the one or more central vessels are almost always six smaller scalariform-vessels (*sc* *); to each of the last succeed a group of quite narrow **spiral** and **annular vessels** (*sp*, *sp* + *a*). The size of the vessels, therefore, diminishes from within outwards, and it is the spiral and annular vessels which lie

* See note on page 144.

outermost. In this the root has relations opposed to those in the stem; a twisting of the ligneous (wood) bundle through half a circle has taken place. The ligneous portions are in this case united into a six-rayed star, and the axial cylinder is therefore described as "*hexarch*." With the ligneous portion the bast portions (*v*) alternate. This last relation is universal for the axial fibro-vasal cylinders of roots. Wood and bast are separated from one another by a layer of parenchymatous ground-tissue cells. The **bast** portions can be recognised by the white shining walls of

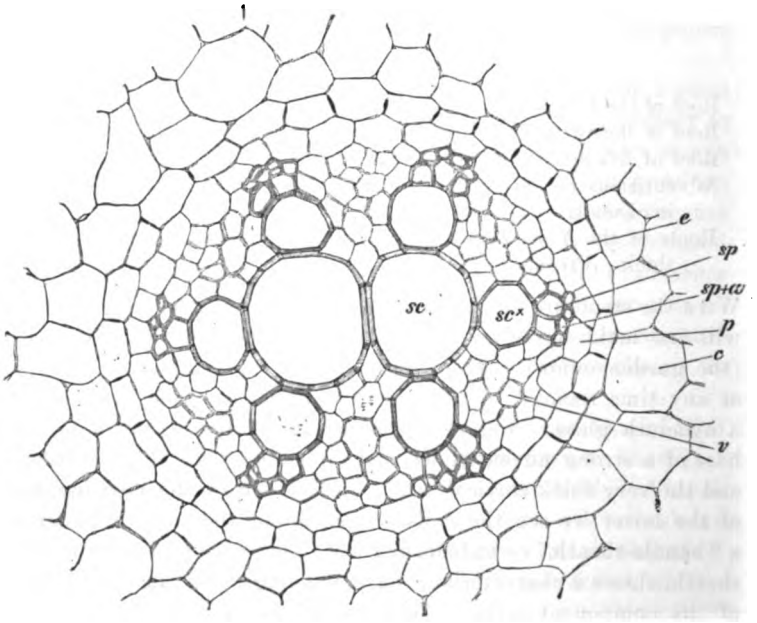


FIG. 53.—Cross-section of the base of a strong adventitious root of *Allium Cepa*. *c*, cortex; *e*, endodermis; *p*, pericambium; *a*, annular vessels; *sp*, spiral vessels; *sc* and *sc*°, scalariform vessels; *v*, bast (× 240).

their cells; they consist of some **sieve-tubes** and companion-cells, which latter are, in the cross-section, not to be distinguished with certainty from the sieve-tubes. From the endodermis the vessels and the bast are separated by a single layer of cells, the **pericambium** (*p*). In concentrated sulphuric acid the entire cross-section is dissolved, with the exception of the epidermis, and the layer of cells impinging thereon, besides the endodermis and the vessels. These last have stained a beautiful yellow. The endodermis,

which during the action of the sulphuric acid will have partially turned over, shows the middle band in the radial walls beautifully undulated. In the outermost cortical layer also, adjoining the epidermis, a similar appearance is, however, to be observed; and if we go back to earlier preparations, we shall become convinced that there also the radial walls show a dark shading. The cells in question are also firmly united together, and form, therefore, a kind of outer endodermis, which has also been termed **epidermoid layer.**[2] The longitudinal section shows the vessels with the thickenings already referred to; and with corallin the sieve-plates of the sieve-tubes can easily be made visible through their staining rose-red. From the sieve-tubes their companion-cells can now be distinguished by their abundant contents and their smaller length. The waviness of the middle band of the radial walls of the endodermis, seen in surface view, shows as a ladder-like thickening. The pericambium cells have the same form as those of the endodermis, but greater length. It will be noticed that the inner endodermis (bundle sheath) takes up corallin into its cell-walls comparatively greedily, while the outer endodermis, on the other hand, is prominent in the surrounding tissue by its want of colour.

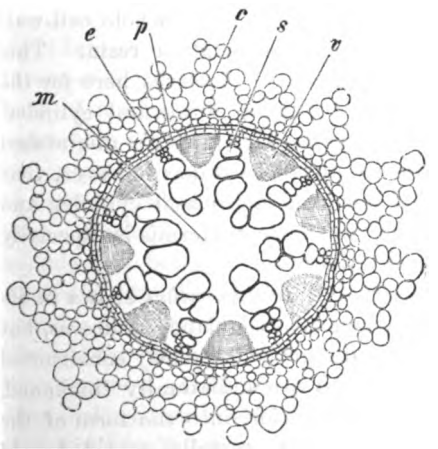


FIG. 54.—Cross-section through the root of *Acorus Calamus*. *m*, pith; *s*, wood; *v*, bast; *p*, pericambium; *e*, endodermis; *c*, cortex (× 90).

The root of *Acorus Calamus* [the sweet flag, not uncommon by the sides of streams and ditches in the eastern and midland counties, and the root of which is used in perfumery] will give us further information on the subject of root-structure. The cross-section of a piece of a fully-developed root (Fig. 54) shows that here the fibro-vasal rays (*s*), *i.e.*, the ligneous portion of the axial cylinder, are not combined in the centre of the cylinder. They are usually, to the number of eight, arranged in an unbroken ring, while the middle is occupied by a **pith.** The large vessels

lie, as in *Allium*, towards the interior, the small ones towards the periphery. The bast (*v*) alternates, as usual, with the wood rays. They are separated laterally by a single or double layer of parenchymatous ground-tissue cells, and outwardly are separated from the endodermis by a unilamellar pericambium (*p*). The endodermis consists of flattened, thin-walled cells. The endodermis, the pericambium, and all the other ground-tissue cells in the axial cylinder, are usually closely filled with starch; thereby the bast-portions, from containing no starch, show up specially clearly in the figure. The cells of the inner cortex are separated into unilamellar layers by numerous air canals. In the periphery the cortical cells are crowded together into a firm, strong, multilamellar sheath. The outermost, hypodermal cortical layer consists of radially elongated cells, and here, as in other roots, forms an outer endodermis, which persists, whilst the epidermis itself dies and is destroyed. If potash solution is run in, the starch disappears out of the cells, and the existence of dark shadings on the radial walls of the endodermis can be clearly determined. On the inner endodermis, as treatment with sulphuric acid shows, only the band which forms the shadings is cuticularized, while of the outer endodermis the whole cell-wall is. The cells of the outer endodermis contain **resin.** This endodermis has a mechanical significance; it serves here for the protection of the surface, and of the axial fibro-vasal cylinder. Through the cuticularization they have acquired a diminished extensibility and an increased solidity. In order, however, that the passage of fluids between the axial fibro-vasal cylinder and the cortex may remain possible, the inner epidermis is especially cuticularized only on the radial walls.[3]

A cross-section through the root of *Iris florentina* shows in its axial fibro-vasal cylinder the greatest resemblance to *Acorus*, but in it, on the other hand, the endodermis is differently constructed (Fig. 55). The cells themselves (*e*) are unilaterally thickened, always on their side towards the interior, into the form of the letter U, and the thickening mass is beautifully stratified. At isolated points an unthickened cell occurs, and it can be determined that, whenever present, such a cell (*f*) always lies in front of a ray of the wood. These cells have been called "**passage cells.**"[4] They are permeable, and maintain the union with the surrounding cortex (*c*). In concentrated sulphuric acid the thickening layers of the endodermis swell and are dissolved; only the cuticularized

middle lamellæ, forming a delicate layer around the endodermis cells and also around the passage cells, remain. Similarly, the middle lamellæ between the vessels and in the pith are not dissolved, and form a delicate, brownish-yellow network. A tangential longitudinal section, which skirts the endodermis, shows us that the longitudinal stripe of this endodermis which lies outside the wood portions consists of an alternation of long thickened cells and of short, unthickened passage-cells, with abundant cell-contents. Here and there two such passage-cells follow one another.

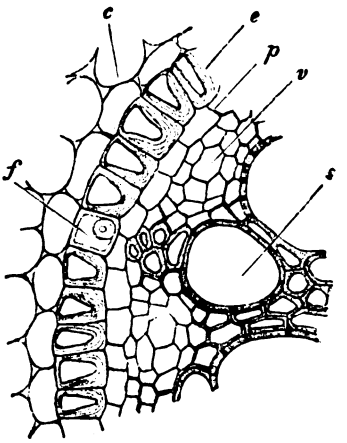


FIG. 55.—Part of a cross-section through the root of *Iris florentina*. *e*, endodermis; *p*, pericambium; *f*, passage-cells; *v*, bast; *s*, vessel in the wood; *c*, cortex (× 240).

The roots of the dicotyledons are less favourable for study than those of monocotyledons. After we have obtained an insight into these latter, it will not, however, be difficult correctly to interpret the former. We first prepare a cross-section from the base of a strong adventitious root of the runner of *Ranunculus repens* [the creeping buttercup, abundant everywhere by roadsides and in pastures]. The axial fibro-vasal cylinder does not appear so sharply defined towards the cortical tissue as it is in monocotyledons. With careful observation, however, we find here also, at the boundary of both, the endodermis marked with its dark shadows. According to the strength of the root, the wood in the axial cylinder is in four or five rays; the great vessels here also lie towards the interior, the small ones outwardly. In monocotyledons the innermost vessel is often distinguished by its special size; in dicotyledons this is seldom the case, and is not to be observed in *Ranunculus*. The wood rays in *Ranunculus* reach the centre of the cylinder, and here amalgamate more or less completely with one another.* Yet, if at all, the innermost vessels are only completely formed quite late, and remain mostly in the form of thin-walled,

* In the adventitious roots upon the rhizome of *R. repens*, according to de Bary, *Comparative Anat.* (Eng. trans., p. 365–6, and Fig. 165), the axis of the cylinder is occupied by one large vessel or pitted duct. [ED.]

elongated cells. The bast bundles alternate with those of the wood in the customary way.

The roots of the vascular cryptogams are simpler, but are constructed on the same type as are those of the phanerogams.

The processes which lead to the secondary increase in thickness of those roots of Dicotyledons and Gymnosperms which are capable of it, we will follow out in *Taxus baccata* (the Yew). For this purpose we procure a piece of root with young uninjured branchlets. We take a cross section through a root about $\frac{3}{8}$ inch from the tip. The surface of it is composed of a parenchymatous cortex at least ten cells thick. The outermost cell-layer of the cortex is not sharply limited, as a true epidermis is wanting. The centre of the section is occupied by the axial fibro-vasal cylinder. This is surrounded by an endodermis. This consists of flattened, thin-walled,* corky cells, whose walls are brown, and whose diameter is manifestly like that of the cortical cells. These cells show, on the radial walls, the characteristic dark shadings. Around the endodermis is developed a "**strengthening layer,**" likewise unilamellar; its cells have the width of the other cortical cells, but are, however, distinguished in their radial walls by a thick, shining, yellow ring. These annular thickenings correspond in adjoining cells, and therefore give in cross-section the figure of a bi-convex lens. The axial fibro-vasal cylinder shows a diametrically-placed, diarch, ligneous body. At the opposite ends of this stand the narrow, dark-looking spiral vessels. Further inwards from these extends a band of the tracheïdes, with bordered pits, of the kind characteristic of Coniferæ. They can be readily recognised by their clear yellow, strongly-thickened walls. The tracheïdes, extending inwards from the two groups of spiral vessels, are amalgamated firmly together into a single straight plate in the midst of the fibro-vasal cylinder. On each side of the tracheïdes lies a, in the main, two-layered band of ground-tissue cells, with narrow cavities, thin walls, and containing starch. To these adjoin the still smaller-celled tissue of the thin-walled bast. Finally, we find on the other side of this last a sheath, about four layers thick, of larger starch-containing cells. These last cells close together into a complete circle, which, against the spiral vessels, appears greatly reduced; this represents the pericambium.

* The unthickened walls of the endodermis cells of most dicotyledonous roots stand in manifest relations with the general power of increase in thickness of their axial fibro-vasal cylinder. [Ed.]

Now examine a cross-section at about 1 inch from the tip, and we shall see that on both sides of the plate of tracheïdes the layer of the ground-tissue, impinging on the bast elements, has commenced to divide. It is converted into a streak of **cambium**, which henceforth cuts off internally tracheïdes, externally bast, and on both sides medullary rays. We will take a view of the further activity of this strip of cambium further from the tip, and at the same time inform ourselves as to the subjoined Figure, 56. The

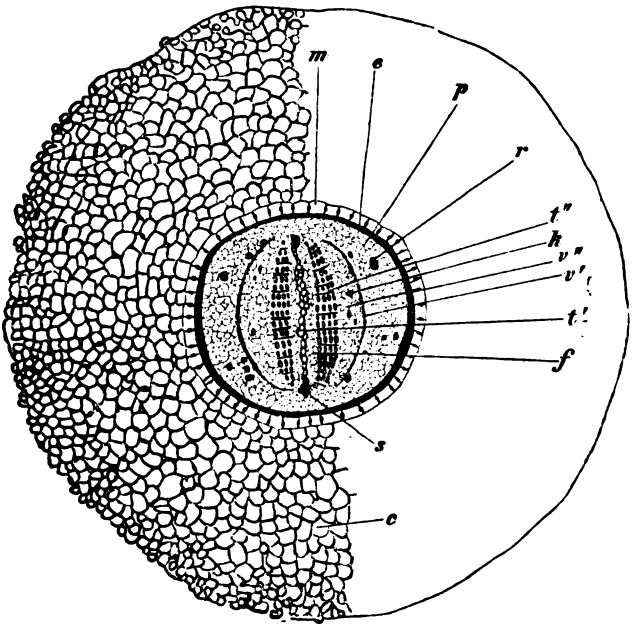


FIG. 56.—Cross-section of a root of *Taxus baccata*, after the commencement of its increase in thickness. *c*, cortex; *m*, strengthening layer; *e*, endodermis, or bundle-sheath; *p*, pericambium; *s*, spiral vessels; *t'*, primary band of tracheïdes; *f*, bands of ground-tissue; *t''*, secondary tracheïdes with medullary rays; *v''*, secondary bast; *v'*, crushed primary bast; *k*, cells in the secondary bast with crystals in their walls; *r*, resin-containing cells in the pericambium (× 42).

cross-section shows first the relations already known to us; the cortex (*c*), which, however, has lost the hairs from its outermost layer of cells; the outer strengthening layer (*m*), the endodermis (*e*), and the axial cylinder. The outermost cell-layer of the pericambium has in the meantime begun to divide by tangential walls, and changed into a still thin **periderm**. On both sides of the plate of tracheïdes (*t'*) we see the inner inactive layer of the

ground-tissue (*f*), the so-called "**connective tissue**"; further on the newly-formed and radially-arranged tracheïdes (*t''*), with numerous interpolated medullary rays. It is easier to obtain information as to these relations if a little potash solution is added to the preparation. The vessels (*s*) at the ends of the central plate stand out clearly, with dark outline. The central plate of tracheïdes (*t'*), as well as the secondary tracheïdes formed by the cambium (*t''*), are stained a beautiful yellow; the connective tissue remains white. The wood bands, secondarily developed, have a plano-convex outline; at their ends they run out into points, but do not as yet join outside the vessels. At the outer margin of the ligneous body we find the cambium, and outside that the secondary bast (*v''*), which after the action of potash appears white, but in which, however, single cells (*k*) appear black. These are the cells in the walls of which crystals of oxalate of lime have become embedded. The primary bundles of bast (*v'*) are found crushed on the outer side of that which is secondarily produced. In the pericambium, after potash, far more clearly than before, single indefinite cells show up by their yellow-brown contents; they contain resin. The cork-layer, developed from the outermost pericambium layer, is coloured yellowish-green by the potash, the thickening rings of the strengthening layer a bright yellow. The endodermis is crushed by the cork-layer.

We will still further examine the cross-section through a root about $\frac{1}{12}$ inch thick, which has already cast off its cortex, and shows a dark-brown surface. The cross-section shows us a completely closed woody body, and the figure of it would not be distinguishable from that of a cross-section of a stem of similar thickness, were it not that the place of the pith is occupied by the primary plate of tracheïdes. The vessels at the edge of this plate are now difficult to recognise. The plate is enclosed in the starch-containing connective tissue, which here compensates in a way for the medullary sheath, and into which the oldest medullary rays open. The two ligneous bodies have joined in front of the groups of vessels, and the medullary rays at these places are hardly longer noticeable for their special width. The surface consists of the annular, closed cork-sheath, produced by the outermost layer of pericambium. The secondary bark consists of the secondary bast and the elongated medullary rays; the tissue here representing the primary cortex is formed from the enlarged and partially multiplied cells of the pericambium, filled thickly with starch.

Longitudinal sections through these roots are of interest in so far that we first with the aid of such determine that the central plate of tracheïdes consists of just the same elements as the secondary wood. We again find the spiral vessels at the edges of this plate, and determine that the cells of the endodermis have only small height, while those of the strengthening layer are far larger, and even surpass in height the contiguous cells of the cortex. With corallin the tracheïdes stain, alike in cross and longitudinal sections, a beautiful corallin-red, and the sieve-plates stand out in the primary and secondary bast. The rings of the strengthening layer also eagerly take up corallin.

Dicotyledonous roots, as a rule, have stellately arranged wood portions, instead of the two only which are present in the Yew. In all which thicken the same essential plan is, however, carried out; streaks of cambium appear on the external side of the wood opposite to each portion of bast; gradually the cambium streaks extend and join into a ring which encloses all the primary wood and excludes all the primary bast, and forms wood internally, bast externally, and medullary rays on both sides. In some cases the medullary rays formed opposite to the primary bundles of wood remain far broader than the others, and the ligneous mass continues to be rayed, the broadening rays, however, being alternate to the original rays of wood; but as a rule these medullary rays are not distinguishable from the others, and the wood is formed in rings, with the cambium, and outside that bast, just as it is in the diarch fibro-vasal cylinder of the root of the Yew.

NOTES ON CHAPTER XII.

[1] De Bary, *Comparative Anat.* (Eng. trans.), p. 351; there find the older literature; Olivier, *Ann. des Sc. Nat. Botanique*, Ser. VI., Bd. XI., p. 5, etc.

[2] Compare v. Höhnel, *Stzbr. d. kais. Akad. d. Wissensch. in Wien, math. naturwiss. Cl.* Bd. LXXVI., I. Abth. 1877, p. 642; Olivier, *l.c.* [This is called the "suberose layer," by Van Tieghem, *Traité de Botanique*, p. 686. The "pericambium," in reference to one of its usual functions, has been styled the "rhizogenic layer."]

[3] Schwendener, *Abh. d. kgl. Akad. d. Wiss. in Berlin*, 1882. *Die Schutzscheiden und ihre Verstärkungen.*

[4] Compare herewith Schwendener, as above, p. 13.

[*Note to page* 136.]

[a] In old roots the endodermic cells are thickened on their inner and radial walls in U-form, and these thickening layers disguise the original structure of the radial walls.

CHAPTER XIII.

THE VASCULAR BUNDLE OF THE FERNS AND LYCOPODIACEÆ [CLUB-MOSSES].

MATERIAL WANTED.

Leaf-stalks (base of) of the Bracken fern (*Pteris aquilina*). Fresh, or in alcohol.

Leaf-stalks of the Polypody fern (*Polypodium vulgare*). Fresh, or in alcohol.

Leaf-stalks of the Hart's tongue fern (*Scolopendrium vulgare*). Fresh, or in alcohol.

Stems of Club-moss (*Lycopodium*, sp.). Fresh, or in alcohol.

WE will now make ourselves acquainted with the structure of the vascular bundle in the stem and leaves of the Ferns. The vascular bundles are here constructed **concentrically**, whereby the wood is completely, or almost completely, invested by the bast. As object of investigation we select *Pteris aquilina* [the well-known Bracken fern]. The relations of the vascular bundles are here the easiest to master, although the object, on account of the numerous sclerenchyma fibres in the ground-tissue, is not exactly a good one for preparation. It is best to cut the rhizome close behind its growing end, or the leaf-stalk [*rachis*] of a leaf that is still young. In such sections the vascular bundle will be found already fully developed, while the characteristic thickening of the ground-tissue is still wanting. The structure of the vascular bundle is the same in rhizome as in leaf-stalk, and should serve to illustrate the accompanying figure, 57, which shows us the cross-section of a vascular bundle from the base of a leaf-stalk. On account of the space which they occupy, a small bundle has had to be selected for representation; still, all the elements entering into its composition can be sufficiently well represented in the figure. The first things to strike the eye are the great **scalariform vessels**; but the lesser vessels are thickened in the same way, and only the small protoxylem

elements, placed somewhat laterally to these smaller vessels, and on the concave side, show a spiral thickening. Where the vessels are not in contact, they are bounded by flattened starch-containing cells (*lp*), which, here also, we can designate wood-parenchyma.[1] Vessels and wood-parenchyma together form the wood, which is almost enclosed by the bast. The elements of the bast bounding the wood are the sieve-tubes (*v*), and to these

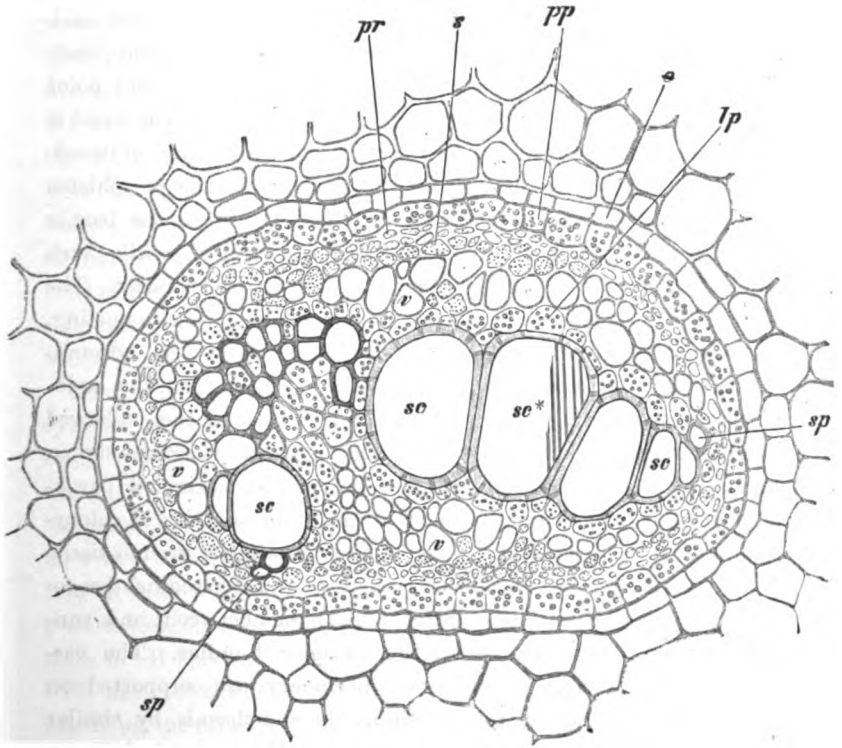


FIG. 57.—Cross-section through a vascular bundle from the leaf-stalk of *Pteris aquilina*. *sc*, scalariform vessels; *sp*, small scalariform (often mistaken for spiral) vessels; in the scalariform vessel *sc**, a piece of a ladder-like wall is broken through; *lp*, wood-parenchyma; *v*, sieve-tubes; *s*, phloëm cells; *pr*, protophloëm; *pp*, periphloëm [bast-sheath]; *e*, endodermis [bundle sheath] (× 240).

[N.B.—The true spiral vessels, or protoxylem, are immediately upon the concave side of the quadrant band of small scalariform vessels situated above the *sp* and *sc* towards the left hand side of the above figure. The larger bundles would have two such protoxylem groups, situated symmetrically about one-quarter from either end (edge) of the bundle.]

follow externally narrower protophloëm elements. These latter elements are rich in protoplasmic contents, which, as iodine proves, are usually wanting in starch. Isolated protophloëm elements are likewise found scattered amongst the sieve-tubes, and bring

about a connection with the somewhat similar wood-parenchyma cells. The periphery of the bast shows a layer of still narrower, thick-walled, protophloëm elements (*pr*). The bast is surrounded by a simple layer, thickly filled with starch (*pp*), which in its position, but not in its origin, shows resemblance with the pericambium, and may be called **periphloëm**. This layer is also known as the **bast-sheath**. This sheath is surrounded by the thin walled, but starch-free and corky endodermis (*e*), which shows the dark shadings on the radial walls. The cells of the periphloëm [bast-sheath] and endodermis correspond with one another, and point to a common origin out of the same mother cells. The wood is bounded at its two edges, first by its covering layer of wood-parenchyma, and then directly by the elements of the periphloëm or the protophloëm. At these two places, therefore, the bast is either completely, or almost completely, interrupted, while such an interruption can, however, be wanting in other ferns. The walls of the endodermic cells are very commonly torn in cutting, whereby the vascular bundle is separated from the ground-tissue. The cells of the ground-tissue bordering on the endodermis are strongly thickened here and there, and then coloured yellow-brown. The cross-section through the rhizome shows, under the deep-brown epidermis, a brown and cutinized parenchymatous tissue, which further towards the interior is colourless and full of starch. This starch-containing ground-tissue is traversed by the fibro-vasal bundles, and by reddish-brown sclerenchyma-fibres. These latter form bands between, and running more or less parallel to, the vascular bundles. The vascular bundles lying towards the periphery are supported on their outer side, immediately adjoining the endodermis, by similar sclerenchyma-fibres, which here represent the **mechanical tissue**. In the interior of the leaf-stalk the relations are similar; but here there is, in addition, a hypodermal ring of reddish-brown sclerenchyma-fibres, which underlies the epidermis. The longitudinal section through the rhizome, or the leaf-stalk, shows most prominently the scalariform vessels. The end walls of these are sharply inclined, with ladder-like bordered pits, partially broken through.[2] On the side-walls, separating the two vessels, it is now easy to determine that the horizontally elongated pits are bordered

on both sides (the **closing membrane** possesses a thickened **torus**). On the wall of those vessels which adjoin a wood-parenchyma cell the border is on the other hand, only unilaterally developed, on the side towards the vessel (the closing membrane has no torus). The longitudinal section may also have cut through one or the other of the spiral vessels, and the sieve-plates of the sieve-tubes may also, but only with most careful examination, be disclosed. We can make the latter somewhat clearer with the aid of corallin, and determine that the terminal sieve-plates are sharply inclined and divided by thickened bands into numerous areas. Besides these, the lateral walls of the tube also bear roundish sieve-pits. Near the sieve-tubes can be recognised the narrow protophloëm-cells with finely granular contents and nucleus; in contact with the vessels, the starch-containing, comparatively short, wood-parenchyma cells. Like to these last are shaped the starch-containing cells of the periphloëm (bast-sheath). The red-brown, long, pointed sclerenchyma-fibres of the ground-tissue show fine pores in their walls.

Comparatively more complicated appears the axial vascular cylinder of the species of *Lycopodium* [club-mosses]. The relations, however, of this will no longer appear so difficult to understand if we study the resemblance of its separate parts to the vascular bundles of the fern. In *Lycopodium* we have, in fact, to do with a combination of numerous vascular bundles, like to these, into an axial vascular cylinder. For investigation, we select *Lycopodium complanatum ;* but another species will serve equally well, since in all species of *Lycopodium* the relations in question recur with unimportant deviations. We somewhat lighten our task by colouring the cross-section at the same time with watery safranin solution. The accompanying sketch (Fig. 58) will serve, however, to give some information. We find in the cross-section of *Lycopodium complanatum* most externally the epidermis (*ep*); then the cortical cells, which first have wide cavities, but further towards the interior diminish in width and increase in thickness, and so form a firm sclerenchymatous sheath, which we will distinguish as the **outer sheath** (*ve*). These strongly-thickened cortical elements, moreover, leave between them small intercellular spaces filled with air. The outer cortical cells have stained more cherry-red with the

safranin; the inner, strongly-thickened ones, more rose-red. The thickened elements of the cortex cease suddenly, and there succeed two or three layers of polygonal cells, elongated somewhat tangentially, and united without gaps, which are coloured cherry-red. These cells have here the position of the endodermis, but they are present in several layers, without the undulated band or the characteristic thickening. On the other hand, like the cells of the endodermis, they are cuticularized, and withstand sulphuric acid well. We will designate this sheath, therefore, as the **inner sheath** (*vi*). Further in follow several layers of equally wide cavitied cells,

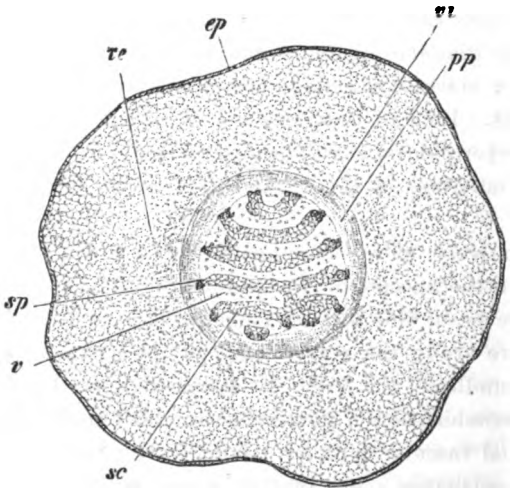


FIG. 58.—Cross-section through the stem of *Lycopodium complanatum*. *ep*, epidermis; *ve*, outer sheath; *vi*, inner sheath; *pp*, periphloëm; *sc*, scalariform vessels; *sp*, annular and spiral vessels; *v*, bast (× 26).

of like diameter with one another in the cross-section, often containing starch, and with walls white and shining, as if swollen. With shorter action, these are not stained; with longer, they are orange-red. These cells are here found in the position of the pericambium, and may therefore, as in the ferns, be called periphloëm (*pp*). We now notice the xylem bands stained beautifully cherry-red. They consist of broad scalariform vessels (*sc*) in immediate contact with one another, *i.e.*, without intermediate cells, and, at the narrow edges, of protoxylem elements, *i.e.*, of narrow cavitied

annular and spiral vessels (*sp*). The ligneous bands in *Lycopodium complanatum* run across the cylinder, and more or less parallel to one another. They are somewhat concave on one side, on the other correspondingly convex; and we can determine, if we take note of the natural position of the rising stem towards the earth, that the bands appear parallel to the surface of the earth, and always with the concave side turned upwards. The small vascular bundles of the leaves, after they have entered into the central cylinder, join on to the spiral-vessel group of a ligneous band, just as in the ferns. The ligneous bands not infrequently anastomose, an example of which can be seen in the lower bands of the sketch (Fig. 58). In the erect stems of *Lycopodium Selago* the whole of the ligneous bands are combined, and form a star. The ligneous bands are surrounded by a single layer of thin-walled, narrow-cavitied cells, which we, as in the ferns, can designate wood-parenchyma cells. At the edges they pass, with their protoxylem elements and wood-parenchyma cells, out to the tissue of the periphloëm. Between the bands formed by the wood lie cells with white, strongly refractive walls; they have narrow cavities, only a middle row is distinguished by somewhat broader cavities. These bands of tissue separating the portions of wood form the bast; the larger elements in this latter are the sieve-tubes (*v*). In specially favourable cases of staining, the walls of the sieve-tubes are rose-red, while the other elements of the bast remain colourless. At the edges of these bands of sieve-tubes the protophloëm elements are distinguished by the narrowness of their cavities. With these protophloëm elements the sieve-tubes reach the periphloëm, the considerably larger cells of which show up clearly against the wood and bast. At the inner limits of the periphloëm, the inner part of the vascular cylinder, consisting of the wood and bast, can be easily broken away in cutting the sections.—The longitudinal sections show us: most externally, the epidermis; then, the broad cortical cells running obliquely towards it; further, the sclerenchyma fibres of the outer sheath; after this, the inner sheath of elongated parenchyma; the periphloëm with white, thicker walls, and cross-walls situated obliquely; the scalariform vessels, and the narrow, in part very greatly stretched, annular and spiral vessels; finally, also, the elements of the bast.

The inner of these, recognisable by their length, somewhat greater breadth, and their poverty in contents, are sieve-tubes. The far shorter, narrow, and much richer protophloëm cells are distinguished by their shining granular contents. With the aid of corallin and aniline blue it is possible, but very difficult, to recognise the comparatively small, inclined sieve-plates.

NOTES TO CHAPTER XIII.

[1 The wood-parenchyma of the vascular bundle of Ferns is very generally designated "packing cells."]

2 Compare also De Bary, *Comparative Anatomy* (Engl. translation), p. 170.

CHAPTER XIV.

CORK,* LENTICELS; THE FALL OF LEAVES.

Material Wanted.

Twigs of the Elder (*Sambucus nigra*) *about* ⅛ in. thick; ditto about ⅙ in. thick. Fresh, or in alcohol. Pretty old twigs of the Laburnum (*Cytisus Laburnum*). Fresh. Fine bottle cork.

Pretty old twigs of the Red Currant (*Ribes rubrum*). Fresh. Base of leaf-stalks, with piece of twig attached, of the Horse-Chestnut (*Æsculus Hippocastanum*). In autumn. Fresh, or in alcohol. Or the same of the Kentucky Coffee-tree (*Gymnocladus canadensis*), the Bastard Acacia (*Robinia Pseudo-Acacia*), or one of the Poplars (*Populus dilatata*).

Strong leaves of *Gymnocladus canadensis*, or *Ailanthus glandulosa*. Fresh. Or of the Ash (*Fraxinus excelsior*) or the Walnut (*Juglans regia*).

We have already, upon various objects, had the opportunity of making ourselves acquainted with the position and structure of cork. None the less will we once again turn our attention to this object, in order to study on the one hand the **Lenticels**, and on the other hand the structure and reactions of the wall of **cork cells**.[1]

Cross-sections through a twig, about ⅛ inch thick, of *Sambucus nigra* (the Elder) show us around the large-celled pith the separate fibro-vasal bundles already bound into a ring by the interfascicular cambium. The cambium ring has also already commenced its activity, and in the fibro-vasal bundles, as well as also between them, has formed in the usual fashion, inwardly secondary wood, outwardly secondary bast. The primary bast appears outwardly supported by sclerenchyma fibres. The cortex is from ten to fifteen cells thick. The projecting ridges of the stem exhibit a strong hypodermal sheath of **collenchyma**, which in the grooves is reduced to a layer two or three cells thick. Under the stomata the collenchyma-sheath is interrupted by the green cortical parenchyma, which here extends to the epidermis. In parts of the stem about ¼ inch thick the for-

* Many rapidly-growing stems show the development of cork with remarkable beauty. One of the best for this purpose is *Hibiscus rosa-sinensis*, a very commonly cultivated hot-house perennial. Fresh or in alcohol.

mation of **cork-layer** commences, always by tangential division of the outermost collenchyma-cells immediately bordering on the epidermis. The inner of the sister-cells thus produced again divides, and it is then the middle cell of the three radially-disposed cells, which further acts as a **cork-cambium** cell. This is easy to recognise, even after the **periderm** has become multilamellar (Fig. 59, *ph*). Outermost in each radial row lies the outer, while innermost lies the inner portion of the original collenchyma cell (*cl*); the flattened cell (*ph*), bounding the inner portion externally, is the **cork-cambium** or **phellogen**-cell.[a] In fortunate cross-sections we can, moreover, determine that the formation of a connected cork-layer is preceded by a peculiar process, which commences under the stomata. The primary cortical cells which surround the air-chamber commence to divide, and the divisions encroach laterally upon the surrounding collenchyma cells. Soon is formed under the stoma a layer of dividing cells in the form of a meniscus (Fig. 60, *pl*), which produces externally colourless cells, which become rounded (*l*), and internally cork-cells, or **Phellem** (*pd*). The outer cells are distinguished as **packing-cells.**[*] They become brown, but not corky; and moreover, as they increase in number, they soon cause such a pressure on the epidermis that this is torn into fissures. In this way is produced the cortical pore, or **lenticel.** If a twig is examined with the naked eye, the lenticels appear as grooves, surrounded by two lip-like cushions. The brown colour of the packing-cells is specially noticeable. On younger parts of the stem the lenticels appear as oval, somewhat projecting spots. Still younger stages

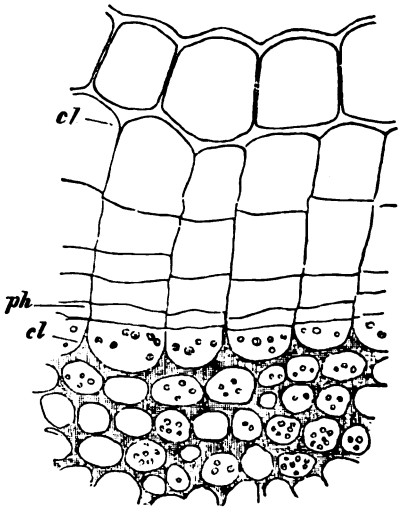


FIG. 59.—Cross-section through the surface of a young stem of *Sambucus nigra*. Epidermis; *ph*. phellogen; *cl* and *cl*, outer and inner parts of the original collenchyma cell (× 240).

[a] See note on page 159.

[*] Füllzellen, translated by Bower & Scott (De Bary, *Comp. Anat.*), as "complementary cells." [ED.]

are marked out by somewhat brighter colour. The section must be taken through such places in order to show the youngest stages of development. Not till after the splitting of the epidermis do divisions begin in the neighbouring collenchyma, which result in the formation of the periderm. The packing-cells of the lenticel are separated from one another; proportionally as they outwardly undergo disorganization, they are replaced by the action of the cambium. The intercellular spaces of the packing-cells are filled with air; between them is communication of the inner tissue of the stem with the surrounding atmosphere. They compensate, therefore, for the stomata in older parts of plants, in which the

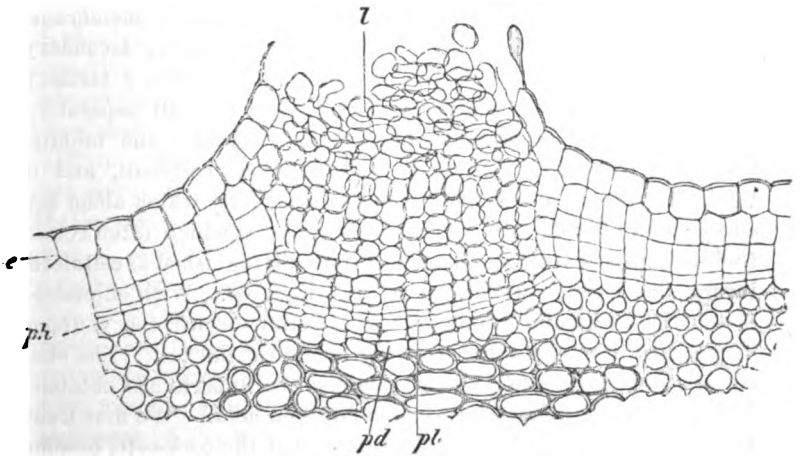


FIG. 60.—Cross-section through a Lenticel of *Sambucus nigra*; *e*, epidermis; *ph*, phellogen; *l*, packing cells; *pl*, cambium of the lenticel; *pd*, phellem (× 90).

cork-formation has begun. For the winter, somewhat more compact and resistant packing-cells are formed. A specially formed closing layer of narrow cells close together is not present in *Sambucus* in winter, while they are met with in many other plants, as also are intermediate layers, which, formed just like the closing layer, are from time to time interposed between the packing cells during the period of vegetation. The cells of these closing and intermediate layers become corky, but allow radially running intercellular spaces between them; so that they do not effect complete closure.[2] In older parts of the stems of *Sambucus* the periderm has longitudinal clefts. These pass through the lenticels, without, however, injuring them. The lenticels persist even on

quite old stems, while the outer layers of periderm between them scale off.

It is recommended to study the structure of cork-cells in the first place upon *Cytisus Laburnum* [the Laburnum], because here they are remarkably thickened. Cross-sections through the cortex of older stems show the **periderm** formed of only one kind of cork-cells. These cork-cells are arranged in regular radial rows. The youngest cork-cells are colourless, the older coloured yellow, the oldest yellow-brown. Those lying at the periphery appear tangentially stretched, often to the disappearance of their cavity. All these cork-cells are greatly thickened, especially on their outer side. In them can be readily distinguished, even without the aid of reagents, the delicate **middle-lamella**, or **primary membrane**, separating the cells, a strong, distinctly laminate, **secondary thickening-layer**, and, on the inner side of this latter, a **tertiary thickening-layer**. Consequently each complete wall separating two cell-cavities consists of five distinct layers:—the middle-lamella, which here represents the primary cell-wall, and is lignified; the two secondary thickening-layers, which alone are corky; and the two tertiary thickening-layers, which often retain their cellulose character and are therefore distinguished as **cellulose-layers**, but here, however, are a little lignified. With chlorzinc-iodine the cork-cells colour yellow to yellow-brown, the younger darker than the older, their tertiary layers the darkest. The characteristic reactions of the cork-material or **Suberin** are obtained by potash, maceration-mixture, and chromic acid.[3] We first treat the sections with potash, and determine that the cork-cells become yellow. We warm the section carefully under a cover-glass upon the object-slide, and find at once that the intensity of the yellow coloration has increased. With the maceration-mixture (chlorate of potash and nitric acid) we obtain a reaction for **Ceric acid.** If unwarmed, the mixture first acts by colouring the cork-cells yellow-brown, besides which all their parts become clearer. If the preparation is now boiled upon the object-slide, if necessary more of the reagent being added, soon of the whole section only the corky layers of membrane remain behind; these finally swell and fuse into a colourless, globular mass. It is the so-called Ceric acid, which is readily dissolved in alcohol, and still more so in ether. If pretty concentrated solution of chromic acid is permitted to work upon the section, of this there finally remains, as before, only the corky layers of the cork-cells. After a

longer time these themselves become so transparent that it is difficult to find them again, although they do not disappear. Notwithstanding that the middle-lamellæ have been dissolved, the secondary thickening-layers adhere to one another.

The **bottle-cork** (of *Quercus suber* [the cork-oak]) consists of almost cubical, thin-walled, comparatively large cells, which gradually pass over into somewhat more strongly thickened, flatter cells, marking the limits of the year's production, to which the cubical cells again succeed. Addition of potash solution colours the section yellow, and first of all the somewhat thicker walled cells marking the year's limits. Upon these it can now be determined that here also each [double] wall consists of five layers, just as we found it in *Cytisus*. Here also the tertiary thickening layer does not give at first the cellulose reaction, excepting after corresponding treatment. The reactions for suberin occur here more beautifully than in *Cytisus*, especially the Ceric acid reaction.

Often from the phellogen are formed not only centrifugal cork-cells, but also centripetal cortical cells, the so-called **Phelloderm.** Rarely, however, does this phelloderm attain such a decided thickness as in the species of *Ribes*. If we prepare cross-sections through older stems of *Ribes rubrum* [the Red Currant], we find under the thin-walled brown cork-layer, first the phellogen, then a thick layer of chlorophyll-containing cortical cells. These last also are arranged in radial rows, which coincide with those of the neighbouring cork. In the inner part of the phelloderm the radial arrangement is lost, in consequence of subsequent extension. The innermost phelloderm-cells border on the collenchyma of the cortex. All the structures proceeding from the phellogen are collected under the term **periderm**; in *Ribes*, therefore, the periderm consists of cork (**phellem**) and cork-cortex (**phelloderm**). It is also of interest to take sections through this year's stem of *Ribes rubrum*, in which the cork formation has for a short time begun. We can here see the first commencement of the phelloderm formation, and at the same time determine that in the plant in question the phelloderm is situated pretty deeply in the cortex. The more external tissue, cut off by the cork-layer from access of sap, perishes, becomes brown, and forms the so-called **Bark.**

The fall of foliage leaves in autumn * results from the inter-

* What follows is a translation of pp. 240–241 of the larger work, *Das Botan-*

position of a **separating layer** [or what we may call an **absciss layer**] which is formed earlier or later during the period of vegetation, and which cuts across the articulation of the leaf-stalk. This absciss layer is the only new formation the existence of which can be proved at the base of the leaflets of a compound leaf, and also at the base of the primary leaf-stalk of many leaves (as those of ferns, and numerous phanerogams); the scar is then somewhat later closed by a cork-layer, or, as in the ferns, by simple drying of the surface cells. In other cases, on the other hand, before the fall of the leaf, is formed at the base of the primary leaf-stalk a periderm, separated from the absciss-layer by a few layers of rounded cells, and which, after the fall of the leaf, is only brought into a state of more active development.[4] We will examine the processes a little more closely in *Æsculus Hippocastanum* (the Horse-Chestnut), during the fall of the leaf. The research is carried on upon alcohol material just as well as upon fresh, so that we can become independent of the time of the year. The absciss-layer, as well as the cork-layer, lie in the position which is clearly visible externally as the boundary between the brown tissue of the cortex and the green tissue of the leaf-stalk; upwards this boundary strikes the angle which the leaf-stalk forms with the bud in its axis. We cut off the leaf-stalk, with the surrounding parts of the cortex, from the twig, and halve it in a median line. We take now a number of delicate longitudinal sections with the razor, in which we take care that some of them also cut through a fibro-vasal bundle. In such longitudinal sections, prepared from fresh material, and examined in water, the cork-layer is at once observable, even with low magnification, as a clear brownish streak, between the deeper brown cells of the cortex, and those of the leaf-stalk. In alcohol-material the cell-walls of the cortex and of the leaf-stalk remain colourless. The cork layer is clearly reddish-brown, especially on the cortical side. It consists of six or eight layers of cells, and joins on to the periderm of the twig with its margins. Its phellogen lies on the side of the stem. This cork-layer is penetrated by the fibro-vasal bundles of the leaf. Separated by some layers of cells from this periderm [and on the leaf-stalk side of it] the absciss-layer, only

ische Practicum, inserted here by request of the Author. I commenced an investigation into this subject, still in progress, in the autumn of 1882, in the Botanical Laboratory at Bonn, under the guidance of the Author. The leaves referred to above were included in that research, and the results, as far as they go, substantially coincide with what follows. [Ed.]

a few cell-rows thick, runs within the roundish cells of the leaf-stalk, recognisable by its yellow colour, the newly intercalated dividing walls, and the more copious contents of its cells, which likewise contain small starch-grains. It is first formed shortly before the fall of the leaf, while the periderm was already present much earlier, and is continued through the living elements of the fibro-vasal bundle. For the rest, the cells of the leaf-stalk are almost completely emptied of reserve food materials; they contain, as treatment with iodine shows, only a trace of starch. In the same way starch is wanting, alike in the leaf and in the cortex, within the fibro-vasal bundle, although in the cortex it is very abundantly represented in the vicinity of the fibro-vasal bundle. The thin-walled elements of the fibro-vasal bundle are, on the other hand, filled with highly refractive masses, which give a tannin reaction. If fresh sections are examined in water, this latter commences very quickly to fluoresce with a bluish tone, from the **æsculin** which comes out of the stem. Numerous cells of the leafstalk contain clusters of crystals, or a single crystal, of oxalate of lime. Preparations treated with aceticized methyl-green show in the cells of the leaf-stalk a remnant of the protoplasmic sac, the nucleus, and chlorophyll-grains. The yellow grains, into which the chlorophyll-grains break up, give to the leaves their autumn tint. The fall of the leaf takes place inside the absciss-layer, the cells of which become rounded, and so disunited; the fibro-vasal bundle is torn through in the corresponding part. The leaf-scar is covered by the roundish parenchymatous cells, which lie between the absciss-layer and the cork-layer, and therefore at first appears greenish. These cells become brown, and dry up quickly in air. The exposed and broken elements of the fibro-vasal bundle wither, and their walls, as well as their contents, become dark-brown. Under these decayed cells a phellogen is now formed also in the fibro-vasal bundle. It arises through division of all the elements provided with living contents. In the vessels which are devoid of a protoplasmic cell-body, the process naturally is interrupted. These, on the other hand, are quickly crushed by the dividing cells. Thus is developed on the leaf-scar, a completely closed cork-layer, which further increases somewhat in thickness. Between the cell-rows of this, the flattened and drawn-out ends of the vessels can later on be still recognised. The dead ends of the fibro-vasal bundles, however, continuously project, to the number usually of 5 or 7, out of the shield-like leaf-scar. As

a specially favourable object for the study of the processes here described, may be mentioned *Gymnocladus canadensis* [the Kentucky Coffee-tree], where it is at our disposal, and also *Robinia Pseud-Acacia* [the common bastard Acacia], or *Populus dilatata*. The results of the investigation will agree in the main with the processes above described. If strong leaves of *Gymnocladus canadensis*, or of *Ailanthus glandulosa* are laid in a damp, dark chamber, the former in about 48 hours, the latter in four days, lose their leaflets on the slightest touch.[5] Longitudinal sections through the place of insertion of their leaves shows that an absciss-layer has been formed at their base. Such an absciss-layer commences its formation also at the base of the common leaf-stalk at about the sixth or seventh day. Under these conditions, however, a periderm is not formed under the absciss-layer. *Fraxinus excelsior* (the Ash) and *Juglans regia* (the Walnut) can also be used in this experiment.

NOTES TO CHAPTER XIV.

[1] For literature see De Bary, *Comparative Anat.* (Engl. trans.), pp. 544 et seq. v. Höhnel, *Stzber. d. math. naturw. Cl. d. k. Akad. d. Wiss. in Wien*, Bd. LXXVI. 1877.

[2] Klebahn, *Jen. Zeitschr. f. Naturw.* Bd. XVII.

[3] Introduced by v. Höhnel, see above, p. 522.

[4] Von Mohl, *Bot. Zeitung*, 1860, pp. 1, 132, 273; Bretfeld, *Jahrb. f. wiss. Bot.* Bd. XII. p. 133; Van Tieghem et Guignard, *Bull. de la Soc. Bot. de France*, 28 July, 1882.

[5] Von Mohl, as above, p. 271.

[*Note to page* 153.]

[6] At first the phellogen gives rise only to cork cells on its outer side; soon, however, it begins, though comparatively but sparely, to cut off cells on its inner side, which contain chlorophyll-grains, and take part in the thickening of the cortex, which the growth in thickness of the stem has stretched. This cortex, developed from the cork cambium, is known as the phellogenic cortex, or *phelloderm*. The whole of the products of the activity of the cork-cambium are collected together under the name *periderm*.

CHAPTER XV.

STRUCTURE OF FOLIAGE AND OF FLORAL LEAVES. TERMINATIONS OF THE FIBRO-VASAL BUNDLES.

MATERIAL WANTED.

Leaves of the garden Rue (*Ruta graveolens*). Fresh.
Leaves of the Beech (*Fagus sylvatica*). Fresh.
Flowers of the Mullein (*Verbascum nigrum*). Fresh.
Petals of the Poppy (*Papaver Rhœas*). Fresh.

WE will now endeavour, by means of a series of examples, to make ourselves acquainted with the structure of leaves. We turn first to foliage leaves, and to kinds which exhibit the smallest amount of differentiation of their inner structure. Our first example shall be *Ruta graveolens* [the garden Rue], the leaves of which also usually remain fresh during the winter. The leaves of this plant are bipinnate, the leaflets ovate. Held towards the light, these leaflets show clear spots; these are the **glands**, filled with **etherial oil**, internal glands in the tissue of the leaf. To the oil contained in them the leaf owes its strong smell when bruised. We take first surface views by means of surface-sections of the epidermis, and determine that the upper side (Fig. 61, *A*) universally has no, or but few, stomata; these, on the other hand, are numerous on the under side (*B*). Elongated pits, filled with air, lead up to the stomata. Above the glands, as can be determined upon either upper or under epidermis, lie usually four cells (*A*, *sc*). These cells form in the centre a shallow depression. In thicker parts of the section, where the glands are not opened by the razor, can be seen in these a highly refractive yellow drop. With still deeper focussing we can determine that under the epidermis of the upper side lies a green tissue of cells, which appear round in optical section (*A*, *p*). These cells are almost completely separated from one another, and the intercellular spaces filled with air. Below the under epidermis are situated cells, likewise green and rounded in optical section, but in much smaller number (*B*, *s*). These cells,

also, are separated by air, and leave, especially under the stomata, wide air-chambers. After obtaining this information, we proceed to cut cross-sections; these we prepare, perpendicularly to the long axis of the leaflet, in the manner already known to us; viz., by placing the leaflet, for the purpose of cutting, between two pieces of elder-pith. The cross-section shows us the leaf-tissue or **mesophyll**, between the upper and under epidermis. Proceeding from above downwards, we see first the epidermis of the upper side (Fig. 62, *ep'*), then a double parallel layer of chlorophyll-containing cells, elongated perpendicularly to the surface of the leaf, which we call the **palisade layers.** We already proved by the surface-section that these cells are laterally more or less com-

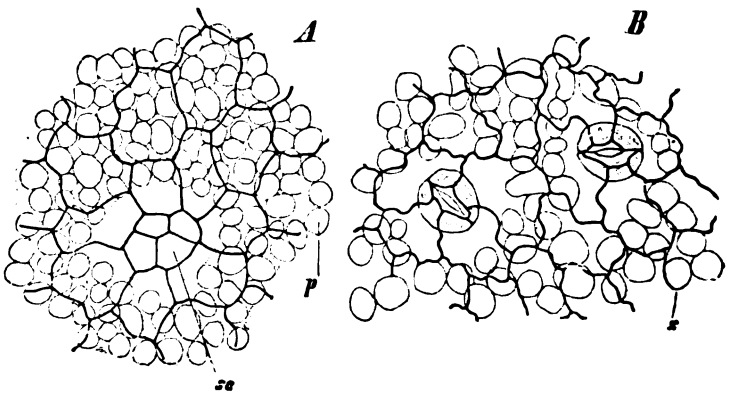


FIG. 61.—Epidermis and underlying tissue of the leaf of *Ruta graveolens*. *A*, epidermis of the upper side; *sc*, epidermal cells over a gland; *p*, palisade cells; *B*, epidermis of the under side; *s*, spongy parenchyma. In *A*, the intercellular spaces, filled with air, are shaded; in *B*, are left clear (× 240).

pletely separated from one another; on the other hand, the two successive layers are closely joined together by their ends. The elements of the second palisade layer (*pl''*) are somewhat less numerous than those of the first; and two of the outer palisade cells often stand upon one of the inner. To these two palisade layers follows a loose tissue, that extends to the epidermis of the under side, and forms a net with wide meshes; this tissue we call **spongy parenchyma**; it contains fewer chlorophyll-grains than the palisade tissue. The cells of the upper layer of spongy parenchyma (*sp'*) are fast joined to the inner palisade cells, each one usually being attached to several of the latter cells. None of the palisade cells remain with their under ends free; where this appears to be

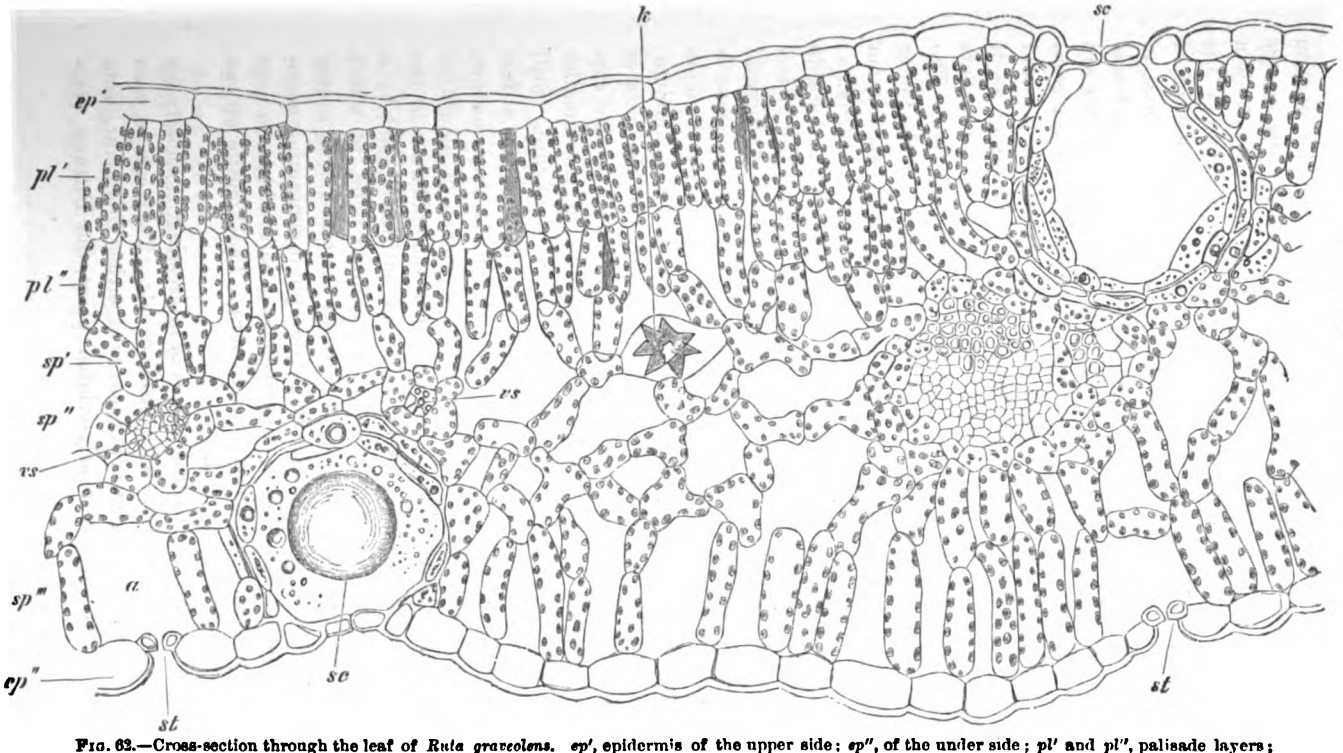


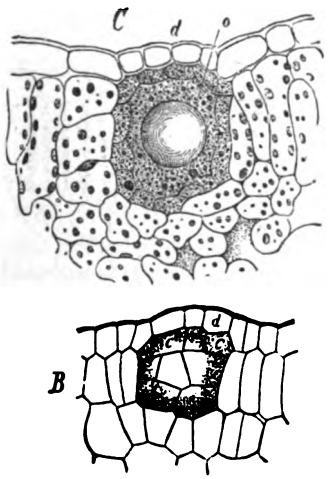
FIG. 62.—Cross-section through the leaf of *Ruta graveolens*. *ep'*, epidermis of the upper side; *ep''*, of the under side; *pl'* and *pl''*, palisade layers; *sp*, spongy tissue; *k*, cell containing a crystal [crystallogenous cell]; *vs*, fibro-vasal bundle; *sc*, gland; *a*, air-chamber [respiratory-chamber]; *st*, stomata (× 240).

the case (as in some of the palisade cells in the figure), the junction does not lie in the plane of the figure. So also in the spongy parenchyma, the cells have no free ends; the ends of all cells join together. The lowermost layer of spongy parenchyma (*sp'''*) is elongated in the direction of the lower epidermis, and directed more or less perpendicularly to it; here, therefore, we have an intermediate formation between spongy parenchyma and palisade parenchyma. The **air-chambers** (*a*) under the stomata (*st*) are left free. Single cells in the spongy parenchyma contain compound crystals of oxalate of lime (*k*). These cells are devoid of chlorophyll, swollen into a barrel-shape, and appear as if suspended between the green cells. At the edges of the leaflets the outer sides of the epidermal cells are strongly thickened. The palisade layer is single at the edges, and passes over on the under side of the leaf into the elongated layer of spongy parenchyma (*sp'''*). The fibro-vasal bundles lie in the spongy parenchyma; the largest, in the **mid-rib** of the leaflet, extends on the one side almost to the inner palisade layer, on the other side to the undermost elongated layer of spongy parenchyma. In the fibro-vasal bundles themselves, we recognise readily the darker-looking vessels and the lighter bast. The radial arrangement of the elements arises from the bilateral but temporary activity of the cambium. Around the bundle is a sheath of parenchyma, the elements of which contain chlorophyll-grains, and which join on to the surrounding spongy parenchyma. The relations of the smaller bundles are similar, as is shown, for example, in the figure. Still smaller fibro-vasal bundles (*vs*), which are reduced to a few vessels and bast-elements, are also met with in the cross-section. These remain to the last surrounded by a sheath of elongated parenchyma. The **glands** (*sc*) impinge upon the epidermis of the upper or under side. They are circular in outline, clothed by a layer of thin-walled, more or less disorganized cells, to which follows a layer of flat cells with granular contents, and pretty thick white walls. The surrounding chlorophyll-containing mesophyll joins on to these cells. The epidermal cells which lie over the gland are flatter than their neighbours. The volatile oil can be readily removed by alcohol.

The mode of origin of these glands is interesting, especially for the purpose of comparison with resin canals. They can be seen well in sections of young growing leaves of either this plant, *Ruta graveolens*, or of another nearly-related plant, the Dittany (*Dic-*

tamnus Fraxinella). It will be readily seen that the gland is **lysigenous** in origin; that is, arises from the breaking down of cells, instead of from their separation. This breaking down commences at the centre of the mass of gland-cells. See Fig. 62*.

Surface-sections at the base of the common leaf-stalk [**petiole**] show the epidermis elongated, and interrupted alike on the upper and under surface by stomata. Oil glands are not wanting. Under the epidermis lies a layer of elongated, collenchymatous cells, and then follows the chlorophyll-containing tissue. In cross-section, the epidermis is seen to be thickened on its outer side, then follows the single layer of thickened collenchymatous cells, this layer being wanting only under the stomata. The two or three layers of palisade-like, elongated, green cells are tolerably similarly developed all round the stalk, but are looser on the under side. To these follow rounded cells, the outer green, the inner colourless, and which get larger more internally. In this inner cylinder of colourless cells run the fibro-vasal bundles, the strongest in the vertical median plane, and nearer to the under side; the others on either side of the large one, and each with its wood portion turned towards the centre of the leaf-stalk. The larger of these fibro-vasal bundles are provided on their external side with a string of sclerenchyma fibres. The activity of the cambium has also apparently lasted longer in these bundles, and it has cut off inwardly secondary wood, and outwardly secondary, thin-walled bast. Only in the inner part of the bundle do we see larger vessels; in the outer portion of the wood are only tracheïdes with bordered pits.



[Fig. 62*.—Oil-cavity below the upper surface of the leaf of *Dictamnus Fraxinella*. *B*, early stage, showing the breaking down of the central cells only; *c* (shaded), cells not yet broken down; *C*, mature state; *o*, a large drop of oil. (× 320, after Sachs.)]

As a second object for investigation, we choose the leaves of *Fagus sylvatica* [the Beech]. On account of the small thickness of the leaf, a thin section is here less easy to obtain. It will be well

to place straight narrow strips of the leaf between the two pieces of elder-pith [or, to pack together several of such strips, and then place them between the pieces of pith]. Only the epidermis of the under side has stomata. Adjoining the epidermis of the upper side (*ep*, Fig. 63), in somewhat radiating groups of cells, is a layer of elongated palisade cells (*pl*). These palisade cells are more or less completely separated from one another by intercellular spaces. At their lower ends they bend together into bunches, and to each bunch is joined one or several funnel-shaped, broadened cells of the spongy parenchyma (*sp'*). These latter are bound together with the elongated cells of the spongy parenchyma into a loose network, which extends to the epidermis of the under side (*ep''*).

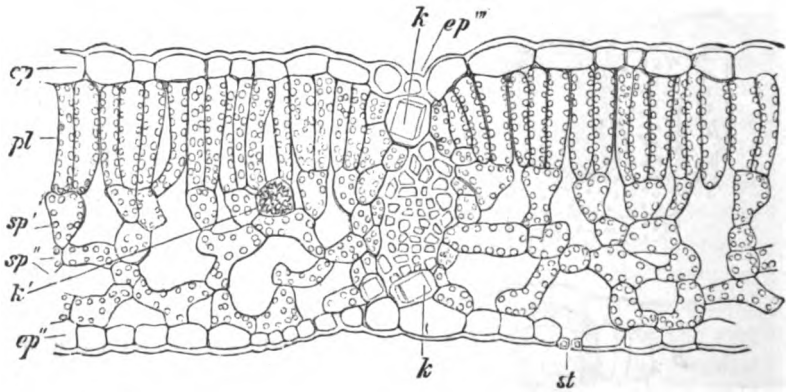


FIG. 63.—Cross-section through the leaf of *Fagus sylvatica*. *ep*, epidermis; *pl*, palisade parenchyma; *sp'* and *sp''*, spongy parenchyma; *sp'* collecting cells; *k*, crystallogenous cells, in *k'*, a cluster-crystal; *st*, stoma (×360).

Single cells, devoid of chlorophyll, but with a cluster-crystal (*k'*), are interposed in the spongy parenchyma. The chief veins, and the lateral veins of the first order, project strongly from the under surface of the leaf in the form of ribs. The projecting part is about as thick again as the other parts of the leaf. The fibro-vasal bundle has its course in the projecting rib. This latter is covered with elongated epidermal cells, to which follow elongated collenchymatous cells. To these adjoin cells, each of which contains a simple crystal; and then follow the multilamellar sclerenchyma-fibres, which ensheath the whole bundle. On the upper side, the palisade layer is interrupted at a narrow part over the fibro-vasal bundle, and is replaced by collenchyma, to which a narrow strip of elongated epidermal cells follows (cf. also at *ep'''*).

A layer of chlorophyll-containing cells surrounds the sclerenchyma sheath, and to these the cells of the spongy parenchyma join on.

The ribs represent the mechanical system of the leaves, which must be constructed firm against flexure.

The ribs may again be, as far as the mechanical development is concerned, likened to girders. The girders are arranged symmetrically with regard to the surface of the leaf, the plane of the girder being perpendicular to this surface. The upper side of the leaf is "stayed" especially against traction, the under side against compression. The girders in this case are arranged, in each rib, in the form of an I, the fibro-vasal bundle forming the "filling." The mechanical capacity of the under part of the girder, constructed against compression, is heightened by its removal as deeply as possible out beyond the under surface of the leaf into the projecting rib of the leaf. By means of the veins the leaf-blade is tightly expanded, and attains thereby the necessary firmness to protect it from tearing.[1]

Smaller fibro-vasal bundles, as those of the figure (63), are protected on the upper and under side only by some sclerenchyma fibres. The ultimate branchlets of the veins are devoid of sclerenchymatous cover, and directly surrounded in their whole circuit by the sheath of parenchyma. The smaller fibro-vasal bundles are accompanied on wood and bast sides by the crystallogenous cells (*k*). Above and under them the epidermal cells are somewhat elongated, and form shallow, depressed streaks. From the epidermal cells upon the veins arise long hairs, like sclerenchyma fibres, which, however, in the fully-developed leaves are mostly thrown off.

It can, without difficulty, be determined that the leaves of the beech have grown especially thick in sunny places, and are so much the thinner in deeper shade.[2] This increase in thickness, as microscopical investigation shows, affects the palisade parenchyma, which can become very considerably elongated and multilamellar. The palisade parenchyma is indeed a tissue specially adapted for strong light-intensity, while the spongy parenchyma is suited for slight intensity. In the palisade-cells we see the chlorophyll-grains only in profile, *i.e.*, distributed over the elongated side-walls, and therefore, according to the intensity of the illumination, only projecting somewhat more or less into the cavity of the cell. In the spongy parenchyma, on the other hand, the chlorophyll-grains, according to the intensity of the illumination, show surface or

profile arrangement, *i.e.*, lie parallel or perpendicular to the upper surface of the leaf. The chlorophyll-grains in the palisade layer are first affected by the sun's rays; while the spongy parenchyma only receives the light weakened by absorption in the palisade-cells. This disadvantage is partially equalized by the surface arrangement possible in the spongy parenchyma. If, however, the intensity of the illumination is too great for the spongy parenchyma, its chlorophyll-grains assume the arrangement in profile. In the Beech leaves which are developed in the most intense sun-light, almost the whole green tissue is formed of palisade parenchyma, while the leaves, somewhere about a third their thickness, which have grown in deep shade, have well-nigh only spongy parenchyma.

In connection with these morphological studies we will enter into a few more physiological conceptions,[3] and test their accuracy by means of the above microscopical structure.

In certain coloured **chromatophores**, and, indeed, in the more highly organized plants, always in the green-coloured chlorophyll-bodies, the assimilation of carbonic acid takes place. Therefore these coloured plasma-bodies only are capable, in light of sufficient intensity, of decomposing carbonic acid gas and water, and out of them constructing combinations rich in carbon. This process takes place to by far the largest extent in the palisade-cells, and these can, therefore, be physiologically designated, as in the highest degree, the **assimilating cells.** The palisade-cells are further, as we have already seen, laterally, more or less completely separated from one another, and come together internally into bundles. The assimilated materials, therefore, are not passed laterally from cell to cell, but rather make their way into the interior of the leaf. Here the bundles of palisade-cells join on to cells of the spongy parenchyma, which often, at the point of junction, are broadened into a funnel form (*sp'*, Figs. 62 and 63), and their function can therefore be that of **receiving** or **collecting cells.** The spongy parenchyma-cells which follow these (*sp''*, Figs. 62 and 63) may, from the same point of view, be designated **conducting cells.** The spongy parenchyma further contains air-cavities, which are in communication with the air-chambers of the stomata; it is, therefore, also a "**ventilating tissue**" [perhaps preferably an **aërating tissue**]. It is also a **transpiration-tissue**, since from the surface of its cells especially copious evaporation takes place into the intercellular spaces. Lastly, the collecting and conducting

tissue is, by reason of its chlorophyll-contents, also an assimilating tissue. The spongy parenchyma joins on to the parenchyma-sheath of the fibro-vasal bundle. To these they ultimately lead the assimilated materials, which are partially conducted in the parenchyma-sheath itself, partly in the bast portion of the fibro-vasal bundle; hence these last together represent the **conducting strings** or **conducting bundles**. The fibro-vasal bundles are, however, at the same time conducting strings for water, which flows in the wood, from this is given off to the surrounding tissue, and is collected in the epidermis, which, in part, functionates as a water-reservoir. It is this conducting tissue of the parenchyma-sheath around the fibro-vasal bundle which, as nerve, or vein-parenchyma,* together with the strongly thickened "mechanical" cells, promoting firmness, forms the tissue of the projecting ribs of the leaf. This vein-parenchyma is continued into the ground-tissue of the leaf-stalk, which, as we have seen in *Ruta*, is mainly composed of conducting (to or fro) and mechanical elements. Assimilating cells play in this only a subordinate part.

We will now make ourselves acquainted with the inner structure of a petal, and also avail ourselves of this favourable opportunity to learn in it the course and termination of the fibro-vasal bundles. The petals of *Verbascum nigrum* [the Mullein] readily permit us to follow the branching of the bundles, and their ends, and to obtain also an insight into the structure of a petal. The air which clings to the bright yellow petal can be easily removed by tapping on the cover-glass. Alcohol cannot here be used, as it makes the structures indistinct. The petal viewed in water shows a delicate epidermis on the upper and under side, and from two to four layers of spongy parenchyma. Only two layers are found at the edge, from which the thickness of the leaf increases till it reaches four layers. The strongest fibro-vasal bundles, as well as the finest branches, reduced to spiral vessels only, are covered by a layer of elongated thin-walled parenchymatous cells. This parenchyma-sheath closes together over

* It will be seen that three terms, viz., rib, vein, and nerve, are used almost indiscriminately by botanical terminologists to represent the same thing. The term *rib* is correct so far as it refers to the mechanical nature of the parts in question, acting just as do the ribs of an umbrella. The term *vein* is correct so far as it refers to the conducting (water and food) function of the fibro-vasal bundles contained in them. How far the term *nerve* may be looked upon as correct in its implication must be left to the future to solve; but under any circumstances, it must be considered far inferior in appropriateness to either of the other two. [ED.]

the ends of the bundles. In the cells composing it protoplasmic movement can be seen. The strongly-branched cells of the spongy parenchyma join on to the elements of this parenchyma-sheath. Very instructive is the view of the ends of the fibro-vasal bundles, which show a radiating junction of the cells of the spongy parenchyma with the sheath.

The petals of *Papaver Rhœas* [the common Poppy] can be likewise studied without further preparation, after the air has been removed by tapping on the cover-glass. Besides the upper and under epidermis, there is here present only one layer of spongy parenchyma. The ends of the fibro-vasal bundles are never free; they join, on the contrary, in connected arches at the edges of the leaf. In their entire course they are surrounded by a unilamellar parenchyma-sheath. To this the cells of the spongy-parenchyma join on from both sides.

NOTES TO CHAPTER XV.

1. Compare Haberlandt, in *Encykl. d. Naturwiss., Handb. der Botanik.*, Bd. II., p. 614; J. von Sachs, *Vorlesungen über Pflanzen-Physiologie*, pp. 59 et seq.

2. Compare herewith Stahl, *Jen. Zeitschr. f. Naturw.* Bd. XVI., 1883; *Ueber den Einfl. des sonnigen oder schattigen Standortes auf die Ausbildung der Laubblätter.* (On the influence of a sunny or shady position on the development of the leaf.)

3. Compare herewith Haberlandt, as above (1), p. 640.

CHAPTER XVI.

THE GROWING APEX* OF THE STEM. DIFFERENTIATION OF THE TISSUES. COURSE OF THE FIBRO-VASAL BUNDLES.

MATERIAL WANTED.

Shoots of the Mare's-tail (*Hippuris vulgaris*). Fresh, or in alcohol.
Buds of a Spindle-tree (*e. g.*, *Euonymus japonicus*).
Buds of the field "Horse-tail" (*Equisetum arvense*). Fresh, or in alcohol.

IT will now be our task, by means of carefully-chosen examples, to become acquainted with the structure of the growing apex. As the first example, we choose a phanerogamous plant, with a very strongly-developed and easily-prepared "growing point," viz., *Hippuris vulgaris* [the Mare's-tail].[1] We take strong shoots for the investigation. From these we cut off the end bud about ¼ inch under the apex of the stem, and first remove from it all the larger leaves. We then hold the bud with the point downwards, flat between the thumb and index-finger, and endeavour to obtain a median longitudinal section of it. For this purpose the razor is passed as perpendicularly as possible between the two fingers in question. First the bud is halved. Each half is cut up subsequently in the same way. Then the section which appears most nearly median is chosen, and in case it does not yet appear thin enough, it is again halved, and so on until a sufficiently thin section is obtained. The operation will at first, perhaps, not be successful, yet in general it presents no insuperable difficulty, and can, at any rate, be attempted. If, however, the difficulty which presents itself cannot be overcome by the beginner, our object can be attained in another way. Instead of between the fingers, place the bud between two flat pieces of elder-pith, and draw the razor between

* Variously known by the terms "growing point," "*punctum vegetationis*," and "vegetative cone." I adopt the term "growing apex" as at once correct, and a suitable complement to the expression "apical growth," in so general use. [ED.]

these. The correct cutting of the object is then, it is true, left more to accident.* Objects which, like the foregoing, have a certain thickness and firmness, can be also clamped between the ends of two pieces of elder-pith, and cut horizontally, together with these, as has been done in earlier cases.

From the sections thus prepared we select one sufficiently median for examination; we recognise it by the slender, regularly-formed **growing apex**. This forms the leaves in **whorls** or circles of many members, and so we see them at a little distance from the apex as isolated protuberances rising symmetrically from the periphery of the vegetative cone. Under the second youngest whorl the **nodes** of the stem begin to be marked as horizontal denser plates [**diaphragms**] of tissue, above and below which, in the cortex of the stem, proceed the **air-passages**. These air-passages, which reach from one nodal diaphragm to the other, are larger in size as the stem increases in volume. The **internodes** elongate very rapidly and symmetrically, and in the same proportion their thickness also increases. Under somewhere about the fourth youngest leaf-whorl begins the formation of vessels in the stem. These are very beautifully seen after the addition of a little potash. These vessels run along the long axis of the stem. They appertain to a fibro-vasal bundle, which grows acropetally, and ends above with some annular vessels. In the tenth to the twelfth nodes are the vessels first visible which appertain to the leaves. These join the vessels of the stem-bundle. In *Hippuris*, therefore, we have to do with a single fibro-vasal bundle, belonging to the stem, and therefore a "**cauline**" **bundle**, with which are

* Another method is as follows:—Cut the bud by as near as possible a median cut into two halves; place the halves in water or alcohol, as the case may be. Examine the cut surfaces, and judge by the regularity of the shape which one includes the actual growing apex, or, if it be a large apex, the most central parts of it. Select this half; stick a needle in a holder through it, well outside the median line, at right-angles to the length of the half, so that the cut surface of the half shall be in a plane parallel with the plane of the needle, and, when upwards, shall have the actual apex to the right hand. With the left hand grasp the needle-holder between thumb and other fingers, but extending the index finger straight out, and flat, so that the curved side of the half-bud lies on and across the finger, and about a third or half an inch from its end. If the needle be lightly pressed upon the finger, the flesh will yield a little, and the object will sink in and be held somewhat firmly, while at the same time the raised part of the finger beyond the object will serve as a good support for the blade of the razor. Holding the razor-blade as flat as possible, take section after section, quite cleanly, until you consider that you have fully passed the central portion of the bud. If in doubt as to which half-bud contains the actual apex, both halves can be treated in the same way. The proper central section must then be selected under a low power. Probably more than one section will be suitable. [Ed.]

articulated the fibro-vasal bundles appertaining to the leaves—the "**foliar**" **bundles.** In the axils of the leaves, at a short distance from the apex, small flat protuberances begin to be raised, which are the commencement of fan-like scales each borne upon a short stalk-cell. Only in specimens in course of flower-production do we here meet with the commencing formation of axillary buds.—In order to become more closely acquainted with the structure of the growing apex, we select a good median longitudinal section, and treat it with *Eau de Javelle* (Potassium hyposulphite). Gas-bubbles soon begin to escape from the preparation. The action must last shorter or longer according to circumstances. The most beautiful results are obtained with alcohol material. The *Eau de Javelle* dissolves out the cell-contents, while the cell-walls stand out sharply. The series of cells are then easy to follow. As soon as the necessary degree of transparency is attained, the preparation must be washed with water. If the section has become too transparent, it can be partially restored by treatment with a solution of alum, or with alcohol. If grains of calcium, which are separated out, should cling to the preparation, dilute acetic acid should be allowed to run in, in order to remove them. The washed preparation can be preserved in glycerine, but must be first laid in very dilute glycerine, and this concentrated slowly in air. In other cases, just as in this, *Eau de Javelle* can be used when it is desired to dissolve the cell-contents, and thus make the cell-walls prominent. Cuticularized cell-walls, after some time, are attacked by *Eau de Javelle.* If the cells are very rich in reserve oily materials, the *Eau de Javelle* offers few advantages.[a] If *Eau de Javelle* is not at our disposal, then treat the section with concentrated potash solution, wash it out, and lay it in concentrated acetic acid. After some time we examine it either in acetic acid or in acetate of potash. It is an advantage not to place the section directly upon the object-slide, but to lay it upon a cover-glass placed upon this, and to cover it with a second cover-glass. We can then, if needed, turn over the section together with the cover-glasses, and so examine it on both sides; we must, therefore, take care that no fluid gets between the under cover-glass and the object-slide.

With pretty strong magnification, we settle, in the first place (compare Fig. 64), that there is a thoroughly definite arrangement of the cells in the "**meristem**" of the growing apex. There are cap-like layers of cells, the separating walls of which form a series

[a] See note on page 182.

of confocal parabolæ. The outermost layer of cells, passing also over the rudimentary leaves, is the initial layer of the epidermis —the **Dermatogen** (*d*). Under this lie four or more undifferentiated layers of cells (**meristem layers**), which appertain to the **Periblem** (*pr*), and from which the cortex of the stem proceeds. Lastly, we find a central cylinder, which tapers conically upwards, ending with usually one cell, and out of which, as can be demonstrated lower down in the section, the axial fibro-vasal bundle of the stem is formed. This tissue we designate the **Plerome** (*pl*). Epidermis, cortex, and axial fibro-vasal bundle, have therefore in *Hippuris* their own special "**histogens**" or **histogenic layers**. There is no single apical cell, though the individual "histogens" of the apex of the growing point may terminate in one or several **initial cells**. Not, however, in all the growing apices of Phanerogams is the separation of the "histogens" so sharply marked as in this case. In many Gymnosperms (Abietineæ, Cycads) a clear separation between dermatogen and periblem does not exist, and often the periblem is not clearly defined from the plerome. In the Angiosperms the dermatogen is always sharply limited, but often there is no limit between periblem and plerome. It is not in any way a question of a differentiation of the tissues which is continued into the meristem of the growing apex, but rather of the mechanical arrangement of the cell-walls, which give to the young tissues the necessary firmness. Very clearly marked in this arrangement is the rectangular junction of the anticlinal walls, *i.e.*, those running perpendicularly to the surface of the apex, and of the periclinal, *i.e.*, those parallel to that surface.[2] For all that, we can retain the terms dermatogen, periblem, and plerome, because the arrangement of the layers of cells, as we have observed it in *Hippuris*, frequently recurs in the growing apex

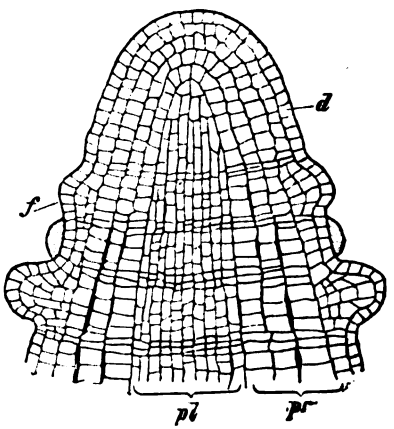


FIG. 64.—Longitudinal section through the growing apex of *Hippuris vulgaris*. d, dermatogen; pr, periblem; pl, plerome; f, commencement of the leaves (× 240).

of Phanerogams. These terms can be therefore conveniently used for denoting definite regions of the growing apex. From the dermatogen, in fact, amongst Angiosperms, if we exclude the very few exceptions, proceeds only the epidermis. The fibro-vasal system is, however, not always traceable in its origin to the plerome, but often to the periblem also. In the earliest rudiments of leaves we see first in the outermost layer of the periblem periclinal divisions set up (at *f*), then follow anticlinal. The dermatogen in the places of protuberance remains unilamellar; it divides only anticlinally. In the same way in the development of buds periclinal and anticlinal divisions take place in the outer periblem layer, and anticlinal only in the dermatogen.

We will now investigate a flat growing apex, as it occurs in most Phanerogams. As an example, we may take *Euonymus japonicus*[3] [the Japanese Spindle-tree], cultivated as an ornamental shrub in many gardens, which can be examined at any time of the year, and the buds of which cut very well. We first prepare cross-sections, in order to obtain a surface view of the growing apex. Treat the sections in this case as we have done with *Hippuris*. With weak magnification we recognise the growing apex as a flat hump, surrounded by the youngest leaf protuberances. These stand in two-membered, alternating whorls, and therefore **decussate** ["opposite decussate"], as we are wont to say. Every new pair of leaves starts, after considerable enlargement of the growing apex, in the gaps present between the preceding pair of leaves (Fig. 65 *A*). With suitable magnification, it is here exceedingly easy to follow the arrangement of cells at the apex. Fig. 65 *B* presents such a figure; an apical cell is therefore not present. Cross-sections taken close under the apex show us a rapidly initiated differentiation of the tissue into **primary pith**, into **procambium**, which will form the fibro-vasal bundles, and into **primary cortex**. The zone of procambium shows in the cross-section a rhombic figure, with somewhat projecting and rounded angles. The procambium consists of thin-walled, narrow, radially-arranged cells. At the angles begins the formation of the elements of the fibro-vasal bundles: protophloëm elements at the outer, spiral vessels at the inner, side of the zone. This region of commencing differentiation of the elements of the fibro-vasal bundles is not defined towards the rest of the procambium tissue. The procambium zone opens at the places where the foliar bundles enter, in order to admit

them. In the axil of each of the young leaves we can see the rudiments of an axillary bud. The form of Fig. 65 *C* is shown by median longitudinal sections, with weak magnification. The flat growing apex, the rudimentary leaves, increasing in size, the axillary buds (*g*), the differentiation of the primary pith (*m*), the procambium zone (*pc*), the fibro-vasal bundles, com-

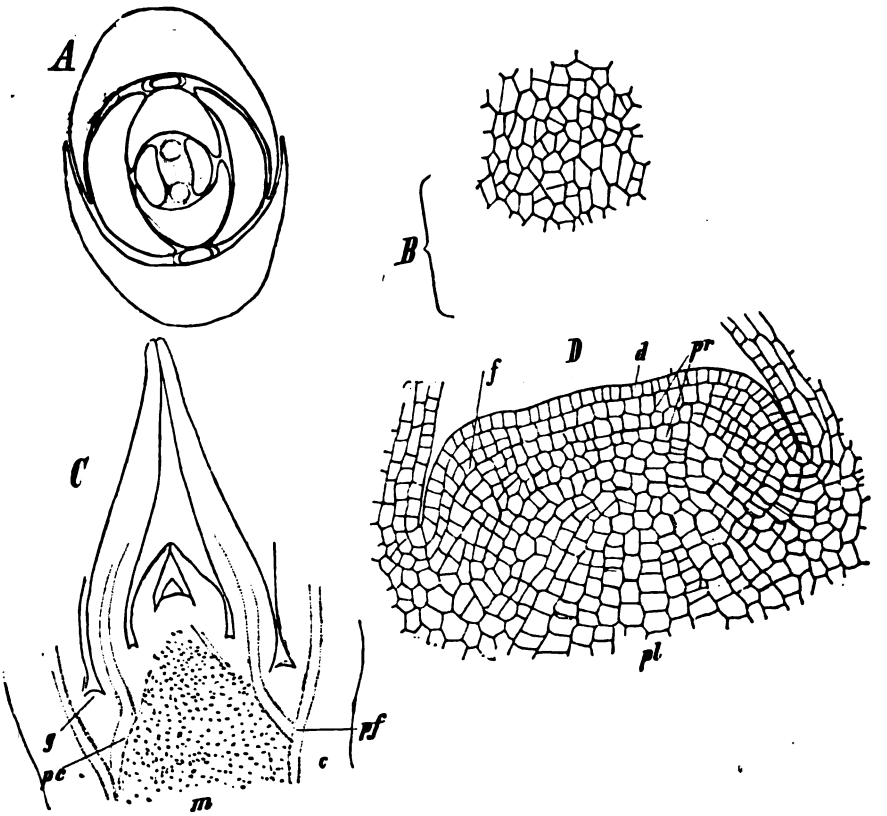


FIG. 65.—Apex of the stem of *Euonymus japonicus*. *A*, apical view of the same (× 12). *B*, apical view of the growing point (× 240). *C*, median longitudinal section through the apex of the stem (× 28). *D*, median longitudinal section through the growing apex (× 240); *d*, dermatogen; *pr*, periblem; *pl*, plerome; *f*, leaf protuberance; *g*, bud protuberances; *pf*, leaf-traces; *pc*, procambium ring; *m*, pith; *c*, cortex.

mon to both leaves and stem [the so-called **leaf-traces**] (*pf*), and the primary cortex (*c*), are to be seen at a glance. Pith and cortex contain great quantities of cluster-crystals of oxalate

of lime. In fresh sections examined in water, the pith and cortex appear greenish, while the procambium-zone appears clear. In order to follow the arrangement of the cells at the growing apex, we again use potash and acetic acid. Outermost on the growing apex we find the unilamellar dermatogen (Fig. 65, *D*, *d*); under that three casing layers, which we have to designate as Periblem (*pr*); and then the central solid cylinder of tissue, which is not everywhere sharply defined towards the periblem, the plerome (*pl*). The growing apex appears very narrow between the two last progressing leaf-protuberances; it is as a rule thus to be seen. On the other hand you have often to cut for a long time before the section passes through the first leaf-protuberances. If you are successful in this, the form presented is that of Fig. 65 *D*. The growing apex appears then much broader, the **histogens** [or **histogenic layers**] can be better followed in it. The formation of the leaves is initiated by periclinal divisions in the two outermost layers of periblem (at *f*); the dermatogen remains unilamellar. Just the same divisions as for the commencement of the leaves take place in the axils of the third youngest pair of leaves, for the formation of axillary buds; the process is likewise initiated by periclinal divisions in the hypodermal layers of cells. It can be determined with certainty that the dermatogen produces only the epidermis, the periblem the cortex, and the plerome the pith of the stem. Less certain is the proof that the procambium ring also proceeds from the plerome. That the formation of fibro-vasal bundles is not exclusively confined to the plerome, follows of necessity from the fact that the part of the fibro-vasal bundle passing into the leaf arises inside the cortex, and therefore out of periblem, and that the entire inner tissue of the leaf, with all its fibro-vasal bundles, is a product of the periblem.

We will investigate lastly a Vascular Cryptogam, growing by means of an **apical cell**, as distinguished from the preceding cases in which growth takes place by an **apical meristem**, and choose as the most favourable object *Equisetum arvense* [the common field "Horse-tail"][4]. It is here comparatively easy to bring into view the apical cell. Shoots in course of development are studied either fresh or by alcohol material. We remove from the apex of the stem a piece about ½ inch long, or rather more, and cut it, as in the above cases, between the fingers, with the apex downwards [or by the needle method described above].

Amongst the longitudinal sections produced we look for one which shows the conical growing apex intact. In order to obtain an insight into the arrangement of the cells of this apex, it is usually necessary to make them more transparent. This may be effected, in this case also, with *Eau de Javelle*, or else by the addition of a little potash. Should this latter act too strongly, and have "cleared" the growing apex, until its cell-walls become unrecognisable, we can remedy the evil by a suitable addition of water. In fresh sections we must avoid the use of any water-withdrawing medium, as otherwise the growing apex

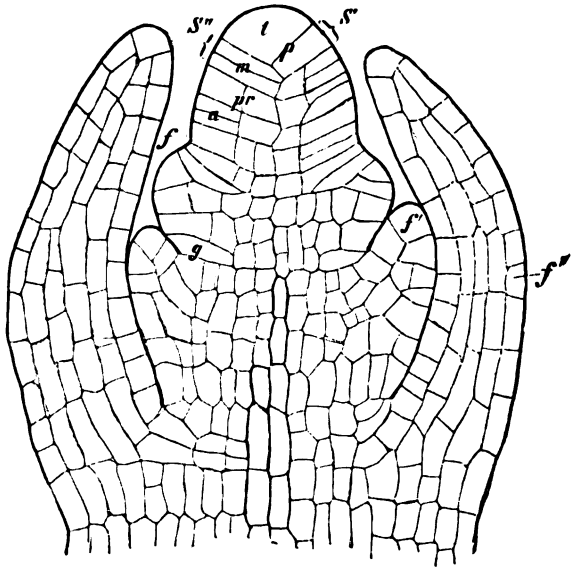
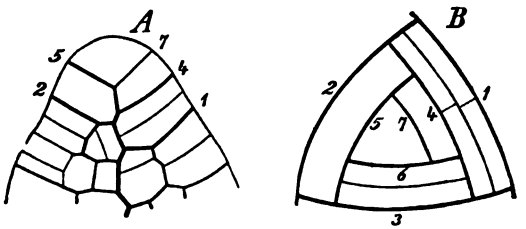


FIG. 66.—Longitudinal section through the growing apex of a main vegetative shoot of *Equisetum arvense*. *t*, apical cell; *s'*, youngest, *s''*, next older segment; *p*, primary wall; *m*, segmenting wall; *pr*, later periclinal; *a*, anticlinal walls; *f*, first; *f'*, second, *f''*, third whorls of leaves; *g*, initial cell of an axillary bud (× 240).

will collapse. Sections of alcohol-material can, on the other hand, be laid in glycerine direct, but not after previous sojourn in water. Sections treated with *Eau de Javelle* cannot be placed at once into concentrated glycerine, but must be placed in very dilute glycerine, which is allowed to concentrate by standing in the air. Sections made transparent with potash can be neutralized with acetic acid, and preserved in acetate of potash. As it is here of special importance to be able alternately to view the

section from its two sides, we lay it, as we have already done in the case of the growing apex of *Hippuris*, between two cover-glasses.

If the growing apex is cut in the proper direction, it presents its apical cell, a three-sided pyramid upon a convex base, in the form of a wedge, the apex of which is sunk in the tissue of the growing point, and its base is arched free towards the exterior. This apical-cell divides by means of partition walls, which are parallel to the existing side-walls, follow one another in spiral sequence, and form segments arranged in three straight rows. These segments (*S*) are shown in profile in our figure 66. They further divide up in definite fashion, and so gradually construct the body of the plant. [The adjoining Fig. 66*, taken from Nägeli and Schwendener's *Das Mikroskop*, will help to further elucidate this process. *A* shows a median longitudinal section of the apex of an *Equisetum* stem, which corresponds pretty closely



[FIG. 66*.—Schemes showing the division of the apical cell in the stem of *Equisetum*. *A*, in sectional, *B*, in apical surface view.]

with that in the text, the figures 1, 2, etc., showing the order of priority of the original dividing walls, cutting off segments from the apical cell. As this cell is three-sided (besides its base), every third wall is not shown in the longitudinal section; in this case walls 3 and 6 are omitted from the figure. *B* gives a diagrammatic view from above of the three-sided apical cell, showing the order of formation of the primary dividing walls, 1, 2, etc. In both figures the lighter lines are intended to show the subsequent divisions of the primary segments.] At some distance from the apical cell, a "bank" is raised upon the growing apex, which grows at its edge with wedge-shaped initial cells. Certain parts of this edge, later on, get in advance in their development, and form the free leaf-apices ["teeth"] of the, lower down, connate whorl of leaves ["leaf-sheath"]. The further removed

from the apical cell, so much the greater are the young leaf-whorls; simultaneously progresses the differentiation of the inner tissue of the stem, especially the separation into denser, small-celled, thin nodes, and less dense, elongated-celled, long internodes (Fig. 67). First the larger-celled pith begins to separate out in the middle of the stem. In the fifth internode, counted from above (in the figure), the first annular vessels become visible in the procambium strings, at the outer limits of the pith, and can be traced from here into the next higher commencement of the leaf-whorl. Each individual fibro-vasal bundle is here common to stem and leaf, and is therefore designated as **leaf-trace**. In each internode just so many fibro-vasal bundles run outwards, as leaves are represented in the leaf-whorl. The leaf-traces, lying at first separated from one another, are, in about the node underlying the seventh internode from the apex, connected by side branches, whereby a complete fibro-vasal system is formed. Approximately in the tenth internode the pith begins to become hollow, through the breaking apart of its cells. In the node, on the other hand, the pith-cells

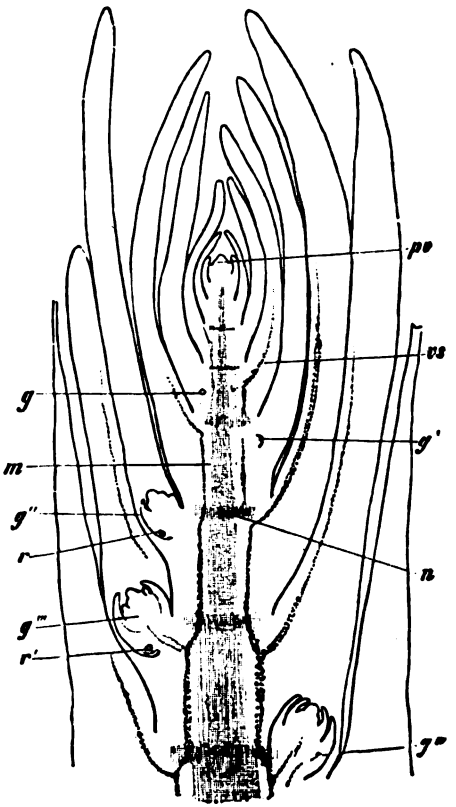


FIG. 67.—Median longitudinal section through a main vegetative shoot of *Equisetum arvense*. *pv*, growing apex of the main axis; *g*, initial for a bud; *g'*, *g''*, *g'''*, *g''''*, stages in the development of such a bud; *r*, *r'*, the origin of a root on the bud; *m*, differentiation of the primary pith; *vs*, spiral vessels making their appearance; *n*, differentiation of the nodal diaphragms (× 26).

undergo a corresponding augmentation, and remain in union.—The lateral buds are initiated by single cells in the axils of the leaf-whorl [leaf-sheath]. They stand in whorls, and, as examination of the mature condition shows, alternate in position with the free leaf-teeth of its leaf-sheath, the tissue of which they finally break through at the base, in order to come outside. The longitudinal section, therefore, shows the somewhat larger buds, grown into the tissue of the leaf-whorl, lying close over the surface of the stem. At about the seventh node, the buds are so far developed that they already possess several embryonic leaf-whorls. Their growing apices can, with care, be used for the study of the apical-cell.

Amongst the Vascular Cryptogams, the Equisetaceæ and Ophioglosseæ possess only **collateral fibro-vasal bundles**, as we can prove readily enough by means of cross-sections through an older internode of *Equisetum arvense*. The fibro-vasal bundles are arranged in a single ring around the hollow pith. In the wood portion, placed internally, of each fibro-vasal bundle, will be noticed an intercellular passage, the **carinal canal**; the thin-walled bast, placed externally, is environed on its sides by the annular and reticulated vessels of the wood. An **endodermis** surrounds the entire ring of fibro-vasal bundles. In the thick cortex, alternating with the fibro-vasal bundles, will be specially noticed broad intercellular passages, the **Vallecular canals**. If we count the free leaf-teeth in the next higher leaf-whorl, we find that the number of the fibro-vasal bundles corresponds with this number.—In order to obtain information upon the course of the fibro-vasal bundles, we now prepare successive cross-sections, so long as to have passed out of one internode through the node into the next internode. We can use for this purpose either fresh or alcohol material; only it is advisable that we use the youngest possible part of the stem, as older parts are strongly silicified, and will quickly blunt the razor. In order to prepare the sections of equal thickness, we can make use of the microtome referred to on page 63. The sections are arranged in proper order upon an object-slide, and can be made more transparent by means of potash. A close comparison of these successive sections enables us to prepare a scheme of the whole course of the fibro-vasal bundles, as in the adjoining Fig. 68, in which we have cut the stem open along one side, unrolled it, and projected the course of the fibro-vasal bundles on the surface of the cylinder thus laid open. We find

that each of the fibro-vasal bundles (*a*, *b*, or *c*), coming down from a higher internode, divides in the node into two forks; and that one of the forks from each of two adjoining bundles combines with a new fibro-vasal bundle, which here enters out of the leaf-whorl (thus the forks from *a* with *b* and *b*, a fork from each of *b* and *b* with *c*). If the fibro-vasal bundles of the lateral buds are already formed, the figure is somewhat complicated by them. Each lateral bud joins (*g*) the vascular system of the parent axis, with two fibro-vasal bundles (*g*), and always with one fibro-vasal bundle on to each of the two forks of the next higher stem bundle, immediately after this divides into its two forked branches. The lateral buds alternate with the fibro-vasal bundles of the leaf-whorl which conceals them, and correspond in their position with the fibro-vasal bundles of the next higher and next lower leaf-whorl.—It follows from our observations that the entire system of fibro-vasal bundles of our stem of *Equisetum* is common; it is formed of leaf-traces, which fork at their base inside the node, in order to join there, through the medium of their forks, with newly entering fibro-vasal bundles. That the leaf-traces combined with one another form the entire fibro-vasal system, is everywhere in vascular plants the most common case; we will therefore limit our studies upon the course of the fibro-vasal bundle to this one simplest possible example.—In the investigation of a more complicated case, it is necessary to arrange the successive sections in the same sequence and direction on the object-slide, in order to be able to compare them more readily. This last task is facilitated if one side of the stem is marked by a shallow longitudinal cut. It is often necessary to draw the successive sections, in order to be able to prove the displacement of the

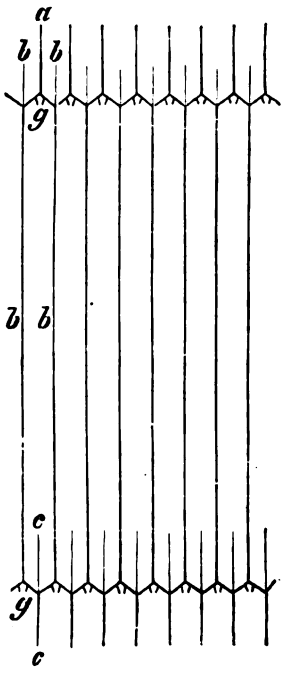


FIG. 68. — Scheme showing the longitudinal course of the fibro-vasal bundles in the stem of *Equisetum*, supposed to be opened out into a plane. *g*, the junction of the bundles of the bud.

individual bundles. Longitudinal tangential sections, made transparent with potash, will in many cases lay bare at once the entire course of the fibro-vasal bundle.

NOTES TO CHAPTER XVI.

[1] Sanio, *Bot. Zeitung*, 1864, p. 223. Note **, 1865, p. 184. De Bary, *Comparative Anatomy* (Engl. trans.), p. 8; L. Kny, *Wandtafeln*, III. Abth., p. 99.

[1a] Noll, *Botan. Centralblatt*, Bd. XXI., 1885, p. 377.

[2] Sachs, *Arbeiten des bot. Inst. in Würzburg*, Bd. II., pp. 46 and 185.

[3] Hanstein, *Die Scheitelzellgruppe im Vegetationspunkt der Phanerogamen*, p. 9. Warming, *Recherches s. l. ramif. d. Phanerogames.*

[4] Compare Cramer, *Pflanzenphysiol. Unters. v. Naegeli*, Heft 3, p. 21. Reess, *Jahrb. f. wiss. Bot.*, Bd. VI., p. 209. Sachs, *Text-Book of Botany* (English translation of Ed. IV. by Vines), pp. 398–402; and Goebel, *Grundzüge*, p. 291. De Bary, *Comparative Anatomy* (Engl. translation), pp. 18, 19.

[*Note to page* 172.]

* If the structures contain starch in quantity, the sections must often lie for days in the reagent in order to dissolve out the grains.

CHAPTER XVII.

GROWING APEX (TIP) OF THE ROOT.

Material Wanted.

Roots of barley (*Hordeum vulgare*), grown in a flower-pot. Fresh, or in alcohol.

Roots of *Thuja* (*e.g.*, *T. occidentalis*), grown in a flower-pot. Fresh, or in alcohol.

Root of fern (*e.g.*, *Pteris cretica*), grown in a flower-pot. Fresh, or in alcohol.

It is desirable now to become acquainted with the growing apex (tip) of roots. We commence with Angiosperms. The structure of their root-apex[1] can be studied with comparative ease in the Gramineæ [grasses]. They provide us, it is true, with only one of the possible types of root-growth amongst Angiosperms, but still one widely spread and instructive, and therefore very suited to give us an insight into the processes in question. In order to obtain favourable material, we choose plants removed with care from flower-pots. If we turn the flower-pot upside down so that the whole contents come out bodily, a result often assisted by tapping the flower-pot lightly on its rim, the root-apices will be usually found free in the exterior of the mass of earth. For careful study we choose the common barley, *Hordeum vulgare*. In order to get general information, we first prepare a cross-section through an older part of the root. In the middle of the **axial fibro-vasal cylinder** we find a large duct or vessel, then in the periphery of it about eight **vascular rays** alternating with the same number of portions of **bast**. As, however, customary in grasses, the vascular rays extend to the **endodermis**, and therefore interrupt the **pericambium**. The endodermis shows more or less clearly the dark radial shadings; to it follows the pretty thick **cortex**. The longitudinal section of the root-apex we prepare between the thumb and forefinger. It must be sufficiently

median; then the structure is plain, even without the use of reagents, but here also *Eau de Javelle* can be used with advantage (cf. p. 172). Above all, it is observable that the body of the root is sharply defined from the **root-cap**. We can, in fact, follow a line, which is prolonged from the surface of the epidermis, continuously

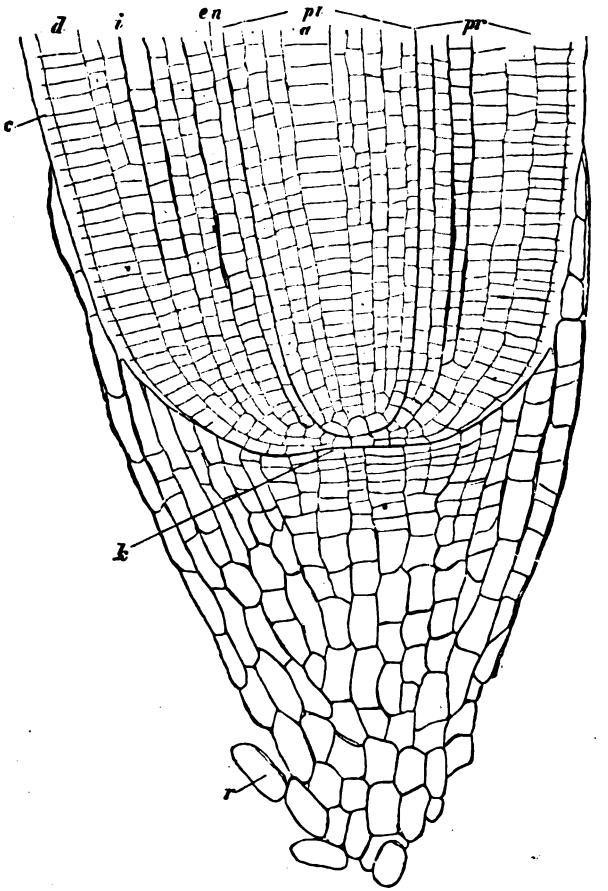


FIG. 69.—Median longitudinal section through the root-apex of *Hordeum vulgare*. *k*, Calyptrogen; *c*, thickened outer wall of the epidermis; *d*, dermatogen; *pr*, periblem; *pl*, plerome; *en*, endodermis; *i*, intercellular passage filled with air; *a*, row of cells which will form the central duct; *r*, disorganized cells of the root-cap (× 180).

over the apex, between the body of the root and the root-cap (cf. Fig. 69). Still the dermatogen does not pass, as such, over the apex, but it must rather be said that the dermatogen (*d*) and the

periblem (*pr*) of the apex come together in common **initial-cells.** In Fig. 68 only one such common initial layer is present, but there may be several. The dermatogen, as such, can be traced up to this initial layer; the periblem also, but one cell thick, merges with it. The plerome, under this common dermatogen-periblem cap, is crowned by a few initial cells. Externally bounding the line which separates the root-body from the root-cap are the initial cells for the root-cap, forming a layer of flattened cells which may be designated the **calyptrogen** (*k*). The cells from the calyptrogen are given off outwardly, and, as the result of their origin, arranged in straight rows; at first flat, they soon increase in height. At the apex of the root-cap they become rounded; finally separate from one another and become disorganized (*r*). It is a peculiarity of the Gramineæ that their dermatogen is strongly thickened on the outer side (*c*). This thickened outer wall is bright white, swells strongly, and appears so much the thicker the longer the section lies in water. At the lateral boundaries of the cells we see highly refractive striæ pass more or less deeply into the thickened outer wall. These are the primary walls of the cells; and the older they are, the more deeply they always penetrate into the thickened wall. These walls clearly show lamination. The periblem has rapidly increased the number of its layers by periclinal divisions. Between its inner cell-layers intercellular passages filled with air quickly put in their appearance, as is represented in our figure by dark lines (*e.g.*, at *i*). The periblem forms the cortex, the innermost layer of which becomes the endodermis.—The plerome ends conically in a group of initial-cells; two such initial cells can be seen in the longitudinal section which we have represented. The plerome forms the axial fibro-vasal cylinder. The differentiation of the large central duct or vessel in this last can be traced from the initial group. The cells from which this duct will be developed are distinguished by their greater breadth (*a*). The elements set apart for the smaller vessels are first distinguishable much later.

The roots of Gymnosperms[3] show, in many respects, a peculiar organization in the meristem of their growing apex. We will study more closely *Thuja occidentalis* The cross-section through a fully-developed root resembles the already-known cross-section of the root of *Taxus baccata* [the Yew], excepting that the roots of *Thuja* are usually **tetrarch** *i.e.*, have four primary ligneous rays, or bundles. The median longitudinal section through the apex

of the root shows a sharply-limited plerome cylinder, which terminates in a few initial cells, and is surrounded by a covering of periblem, some twelve to fourteen cell-layers thick. This last passes over the apex, and forms there its terminal initial layers of eight to ten inner rows of cells, while the outer rows pass over into irregularly arranged, comparatively large cells. These large cells extend to the apex of the root-cap, where they ultimately lose their union, and become disorganized. The root-cap of *Thuja*, and of Gymnosperms generally, consists of the outer parts of the periblem; dermatogen and calyptrogen are wanting. The initial layers of the plerome, passing over the apex of the plerome, divide by periclinal and anticlinal walls. The periclinal divisions increase the number of layers of the periblem, and replace from the interior the elements which are exfoliated from the periphery. The anticlinal walls increase the number of cells in the individual layers, and provide chiefly for the formation of the cortex. As the anticlinal walls in successive layers correspond pretty regularly with one another, they form anticlinal rows of cells, which, straight in the interior, separate from one another externally, like the component rays of spray which collectively constitute the jet issuing from a fountain; and forming therefore a constantly extending series of co-axial parabolæ. The periclinal divisions in the initial layers of the apex have as result that the cell-rows of the cortex, when these are followed towards the point, appear constantly doubled. The most median, straight, anticlinal rows of cells in the periblem of the root-apex are distinguishable from their neighbours. They form a "periblemic column" (Periblemsäule), which is lost in the outermost, brown elements of the root-cap. This column appears clearer, its cells immediately adjoin one another, while those bordering laterally form air-containing intercellular spaces. Moreover, the cells of the column are distinguished by especial richness in starch. As results from the foregoing relations, the root of *Thuja* possesses no epidermis, the outer surface of the root is composed of the, for the time being, outermost layer of the periblem. If such a layer is followed in the direction of the apex, we shall soon see it pass under another, which for a time provides a surface. The outermost living layers of cells are protected at their surface by the collapsed walls (become brown) of the disorganized layers of cells. The roots of the Gymnosperms have, in general, no **root-hairs**; we search for such in vain in *Thuja occidentalis*. The adjoining figure, 70, gives, with low magnification,

the structure of a longitudinal section, and can facilitate our obtaining information about it. Naturally, the arrangement of the cells can only be indicated with such a low magnification. Passing from the exterior towards the interior, we see, therefore, the brown, collapsed covering of cells (*x*); then the periblem (*pr*), which can be traced over the apex of the root, and whose outermost layers, therefore, form the root-cap; lastly, the plerome (*pl*), the termination of which is not quite clear with low magnification. We are inclined to imagine that the upper part of the plerome is larger than it really is, because the innermost layers of the periblem, bordering on the plerome, are devoid of intercellular spaces, and therefore (as is shown in the figure) appear just as clear as the plerome cylinder. In the oldest parts of the section the plerome cylinder shows itself surrounded by a red layer of cells, which, as a comparison with the cross-section shows, indicates the endodermis filled with red cell-sap. As we approach the apex these endodermic cells become unrecognisable. Vessels (*s*) also appear in the older parts of the plerome cylinder. The more clearly showing column (*c*) penetrates the apex of the periblem. Upon this impinge laterally the air-containing layers of the periblem. These last, however, reach entirely neither to the plerome nor to the surface of the root. The last is composed of large brown cells.

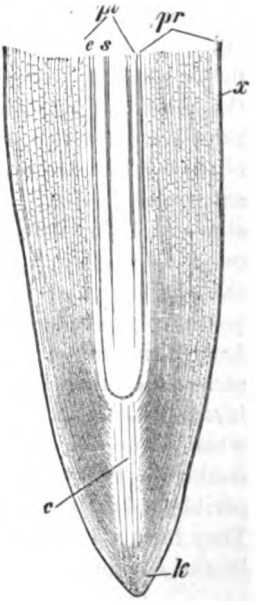


FIG. 70.—Longitudinal section through the root-apex of *Thuja occidentalis*; *x*, outer brown layer of disorganized cells; *pr*, pericambium; *pl*, plerome; *e*, endodermis; *s*, spiral vessels; *c*, periblemic column; *k*, root-cap (× 26).

The roots of Coniferæ will serve to make us acquainted with the methods of branching of roots in general. In the examination of the roots of *Thuja occidentalis*, it will strike us that they bear their lateral roots in four or in three straight rows. We readily prove by cross-sections that three rows of lateral roots indicate triarch *i.e.*, with three ligneous rays, four rows tetrarch, fibro-vasal cylinders. We prepare now a cross-section through a root at the place of insertion of a lateral root, and determine that the lateral root projects from one

of the ligneous rays. As now the ligneous rays run in straight lines in the axial, fibro-vasal cylinder, the arrangement of the lateral roots in straight rows is hereby explained.

We will now endeavour to become acquainted also with the growing apex of a root which grows by means of an **apical cell.**[3] In such roots the same variability as with stems growing by means of apical cells does not exist. The apical cell is always a

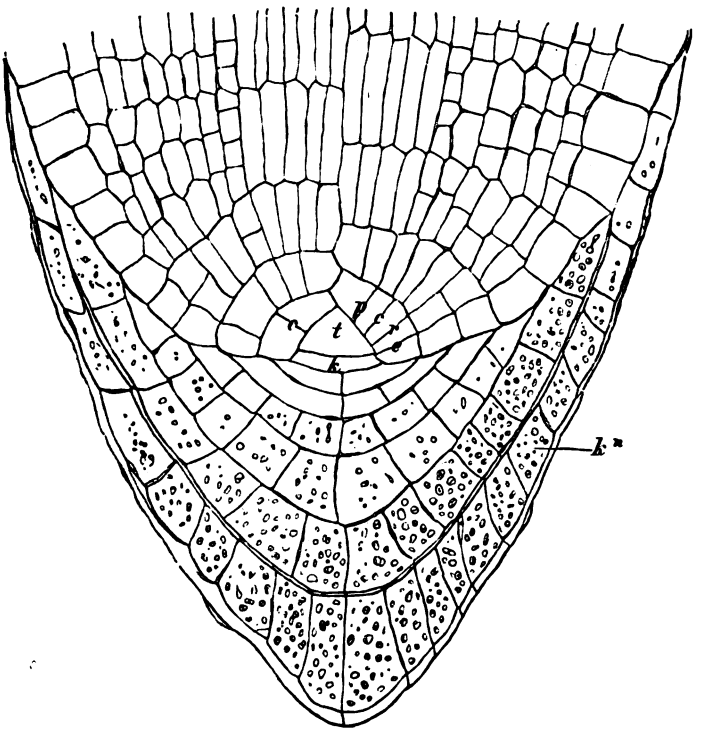


FIG. 71.—Median longitudinal section through the root of *Pteris cretica*; *t*, apical-cell; *k*, initial cell of root-cap; k^a, outermost layer of root-cap; (*e*, wall cutting off epidermis; *r*, ditto cortex; *c*, ditto cambium (procambium); *p*, ditto pericambium or pericycle) (× 240).

trilateral pyramid, and the co-ordination of the segments formed from it remains constant. We investigate the root of *Pteris cretica* (Fig. 71), but can equally well choose any other species of Fern. By turning a flower-pot upside down, we easily obtain uninjured root-apices. The roots of *Pteris cretica*, as of ferns generally, are **diarch**; with the woody portions alter-

nate flattened bast; the pericambium (pericycle) is unilamellar, the endodermis flattened, the cortex become brown, and in its inner part strongly thickened. We now endeavour to obtain, between thumb and forefinger, a thin, median, longitudinal section of the root-apex. It is not very difficult to bring to view the apical cell; it does not here, however, occupy the apex of the root, but is covered by the tissue of the root-cap. This apical-cell (*t*, Fig. 71) has, as in the stem of *Equisetum*, the form of a three-sided pyramid, whose convex base is turned towards the cap, while the apex formed by the junction of the three side-walls is sunk in the body of the root. The divisions, as in the stem of *Equisetum*, take place parallel to the side-walls; besides these however, from time to time (usually after three or four of the above consecutive divisions), a wall parallel to the convex base is formed (compare the figure). The apical-cell retains its form throughout its divisions; the cell cut off from the base has, however, the form of a segment of a sphere. This cell (*k*) is an initial cell of the root-cap, giving to this latter its origin. It divides first by a wall perpendicular to its base into two halves; each half repeats this division, so that four cells, quadrilateral in outline, are formed. In these the division is repeated, and always by walls at right-angles to the original base, so that an older layer of the cap (k^n) consists of a large number of cells. The cells of the older cap-layers are full of starch-grains. They are gradually disorganized while the apical cell cuts off continually new initial cells. The outer walls of the, for the time being, outermost cells of the cap, are strongly thickened. The dividing walls, formed parallel to the side-walls of the apical cell, follow, as in *Equisetum*, the direction of a spiral.

NOTES ON CHAPTER XVII.

[1] Sachs' *Text-book of Botany* (Engl. trans. of 4th edit.), p 147; Janczewski, *Annales des sc. nat., Botanique.* V. Sér., tom. XX., 1873, pp. 162 *et seq.*; Treub, *Musée bot. de Leide*, tom. II., 1876; De Bary, *Comparative Anatomy* (Engl. trans.), pp. 9 *et seq.*

[2] Strasburger, *Coniferen und Gnetaceen*, p. 340; De Bary, *Comp. Anat.* (Engl. trans.), pp. 13 *et seq.*, where see the further literature.

[3] Nägeli and Leitgeb in *Beitr. zur wiss. Bot.*, 4 Heft, 1868, pp. 74 *et seq.*

CHAPTER XVIII.

VEGETATIVE STRUCTURE OF THE MOSSES AND LIVERWORTS

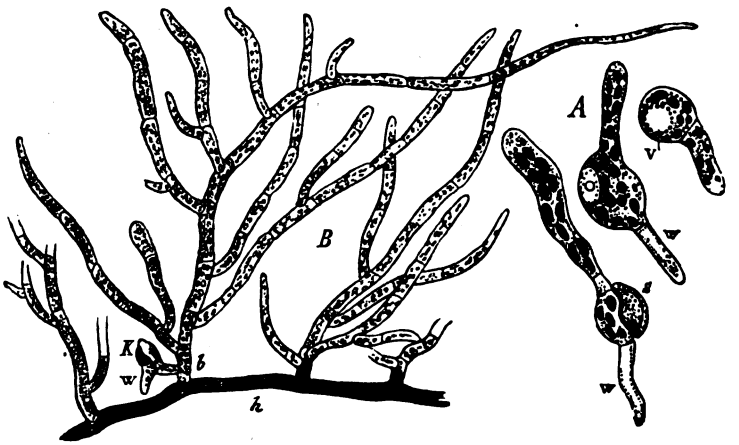
Material Wanted.

Plants of a strong moss, such as *Mnium undulatum*, *M. hornum*, or Polytrichum. Fresh, or in alcohol.
Plants of a Bog-moss (*e.g.*, *Sphagnum acutifolium*). Fresh.
Thallus of a Liverwort (*e.g.*, *Marchantia polymorpha*). Fresh.
Thallus of *Metzgeria furcata*. Fresh.

Hitherto we have studied only the structure of the stem and leaves in **Vascular Plants**; we turn now to the stem and leaves of Mosses, from which vessels are absent.[1] We commence with a comparatively complicated case, where the differentiation of tissue is already somewhat advanced; with *Mnium undulatum*. We first of all prepare delicate cross-sections through the stem. In the midst of the stem appears an **axial cylinder**, composed of narrow thin-walled cells. We can conceive this cylinder as the simplest of all **conducting bundles**. Its cells contain no living contents, only water; they are distinguished from their surroundings by their yellow-brown coloration. To this conducting bundle, which therefore consists of only water-bearing tissue, adjoin the wider cells of the cortex, with greenish-yellow walls, and living chlorophyll-holding contents. At first they increase somewhat in diameter in passing from the interior outwards; at the periphery they become rapidly narrower and thicker walled, and pass over at length, without special limits, into a uni- or bi-lamellar, narrow, strongly-thickened epidermis. At two or three places the outer cell-layer of the stem is prolonged externally into a unilamellar plate of cells, which represents the leaf-wings running outwards from the stem. Cross-sections which are taken from the lower, leafless, strongly-browned part of the stem, show the walls of the peripheral layers of cells coloured dark-brown. From single cells of the surface have grown long, brown-walled, repeatedly branched threads of cells, which here take the functions of roots, and are

distinguished as **root-hairs** or **rhizoids.** These rhizoids, as can be readily seen, are distinguished by obliquely-placed partition walls, which therefore form an exception to the rule, so generally obtaining, of rectangular division. Under numbers of such partition walls, and always under its elevated side, arise the subsequently still more branching lateral branches. Only the apices of the rhizoids which are still growing are provided with colourless walls.

The closest similarity with such root-hairs, from the point of view of the branching and the oblique position of the dividing walls, is shown by the **proembryo** of the typical leaf-bearing mosses, the so-called **protonema,** which is developed from the



[FIG. 71.*—A moss, *Funaria hygrometrica*. *A*, germinating spores; *w*, root-hair, or rhizoid; *s*, exospore (×550). *B*, part of a developed protonema, about three weeks after germinating; *h*, a procumbent primary shoot, with brown wall and oblique septa; from this arise the ascending branches, having limited growth. *K*, rudiment of a leaf-bearing axis with *w*, rhizoid or root-hair (× about 90). (After Prantl.)]

germinating spore. Its branches, however, so far as they do not penetrate into the soil, are colourless, and contain numerous chlorophyll-grains. The **buds,** which develop into the moss-stems, are side-branches of this protonema [compare Fig. 71*]. The near relationship of rhizoids and protonema is shown also in the circumstance, that the rhizoids, if kept damp and exposed to light, can develop a protonema which can give rise to numerous new plantlets. It needs only to lay a turf of *Mnium* with the underside upwards, and keep it damp, in order to produce

numerous green protonema-threads from the rhizoids. This latter in its macroscopic aspect reminds us of terrestrial tufts of *Vaucheria*.

If the cross-section has passed through an injured part of the stem of *Mnium*, the place is seen not to be covered with cork, since this cannot be formed by the Cryptogamia, with the exception of *Botrychium* [the Moonwort fern]; but, on the other hand, the walls of the limiting cells are thickened and browned, and so, apart from their broader cavities, resemble the other surface-cells.

Near the surface can be seen, in the cross-section, isolated small strings of thin-walled cells, which moreover in their coloration resemble the elements of the central cylinder, and which, like them, are without living contents, but on the other hand contain water. These are the conducting bundles belonging to the leaves, which have "blind" ends in the cortex of the stem, while they occasionally, in *Polytrichum*,* join on to the axial conducting bundle of the stem. A leaf, which we examine without further preparation in a drop of water on the object-slide, exhibits a unilamellar lamina, and a multilamellar midrib. This last ends in a terminal tooth, which consists of a number of rhombic cells. The cells of the midrib are elongated, the peripheral cells contain chlorophyll-grains. The lamina of the leaf is unilamellar; it consists of polygonal chlorophyll-containing cells. The broad, seam-like edge of the leaf is formed of elongated, strongly thickened cells. The outermost bear on their edge, at nearly equal distances, one- to two-celled, sharply tapering teeth. Cross-sections through the leaves are obtained at the same time with the cross-sections of the stem. If it is desired to cut cross-sections of separated leaves, which, from their small thickness, is no slight task, it can be considerably facilitated if a considerable number of leaves are stuck together with glycerine-gum, and, without waiting for the gum to dry, the object, thus made thicker, is cut between elder-pith.† The cross-sections are then laid in water, which at once dissolves out the gum. This method can be used at all times, when it is desired to obtain cross-sections of very thin surfaces. Upon these cross-sections of our moss-leaf, we can determine that the lamina is unilamellar, the cells at the

* In *Polytrichum*, the inner part of this conducting bundle is commonly collenchymatous. [Ed.]

† Good cross-sections of leaves can be obtained without preparation by cutting through the crowded leaves at the apex ("bud") of the stem of an actively growing moss, such as *Mnium undulatum*, *M. hornum*, or a *Polytrichum*. [Ed.]

margin of the leaf are strongly thickened. The midrib projects more strongly from the under than the upper surface of the leaf. In its centre, somewhat nearer the under side, lies a string of thin-walled cells, in which we again recognise the **conducting bundle** which we previously saw in the cortex. These thin-walled strings are protected on their under side by some strongly-thickened narrow cells. The structure reminds us not a little of certain greatly-reduced monocotyledonous fibro-vasal bundles, limited to a few bast elements and a weak layer of sclerenchyma.

A withered plant, with the lower cut surface of its stem placed in water, remains withered, but becomes, on the other hand, rapidly turgid if it is immersed with its leaves in water. The admission of water through the leaves is here, therefore, very active.

The structure of the stem of the Bog-mosses offers special peculiarities, and shall therefore be brought here within the range of our observation. We prepare cross-sections of the stem of *Sphagnum acutifolium*. These cross-sections show us a broad central cylinder, which in its interior is constructed of broad, somewhat collenchymatously thickened cells; towards the periphery its cells become gradually narrower, and, in the outermost layers, are coloured yellow-brown. A special conducting bundle is not present in the interior of this cylinder. It is surrounded by a large-celled outer cortex of three layers of cells. The elements of this impinge immediately upon the narrow, yellow-brown cells of the inner cylinder. They are distinguished by large round or oval holes [**pores**] and delicate spiral bands. These pores are easy to see, and that they directly join together the cavities of these cells can be readily proved in places where the sections have cut through such pores. Not infrequently, moreover, fungal threads [**hyphæ**] are seen in these cells, which pass without hindrance from one cell to the other through the pores. These porous elements of the outer cortex of *Sphagnum* contain, moreover, only water or air, and are without living cell-contents. To the plant they serve as a **capillary apparatus**, by which the water may be carried to a place of need. The plants are devoid of cuticularized parts; concentrated sulphuric acid immediately dissolves the entire tissue; comparatively the most resistant are the middle-lamellæ and their junction "seams" of the yellow-brown outer cells of the central cylinder.

The leaf expansion is ovate, entire, unilamellar, and consists, as either surface-view shows, of two kinds of elements. The one are

small, chlorophyll-containing (and therefore also containing protoplasm and nucleus), living cells; and others are dead, filled with air or water, provided with rings or with spiral bands and intermediate open pores. The fact, which must have repeatedly struck us, that dead air- or water-containing cells, so far as they are not strongly thickened, so often need spiral band, rings, or network as thickening of their walls, derives explanation from the circumstance that the said cells are deprived of their turgidity, and need this mechanical apparatus in order not to collapse nor be crushed. The green cells of the leaf-expansion are all joined together, and form a network with elegantly winding walls, each mesh being occupied by one of the empty cells. The green cells serve for the assimilation of carbonic acid gas; the empty cells serve, just as do the corresponding cells of the outer cortex of the stem, as a capillary apparatus for the supply of water. Careful observation shows that the number of pores diminishes towards the edge of the leaf, that they are more prevalent on the under side of the leaf, and stand laterally on projecting cell-walls. The edge itself of the leaf is composed of the narrow green cells, and adjoining these of a single-rowed "seam" of narrower collapsed elements containing watery contents, and slightly thickened on the outer surface. Only the end surfaces of these elements appear to be thickened more strongly, and project outwards proportionally. A midrib is wanting in the leaves, just as is a conducting bundle in the stem; the plants are in this respect, therefore, much more simply constructed than *Mnium;* more complex on the other hand as to the formation of a special capillary apparatus.

The *thallus* of *Marchantia polymorpha* [the common Liver-wort], so widely spread upon damp ground and especially in damp green-houses on the surfaces of the flower-pots, and so readily recognisable by its **bulbil** or **gemmæ-cups**, and ultimately also by its more rarely produced disk-like or umbrella-like **receptacles**, shows a tolerably complicated structure. The absence of cormophytic development does not therefore necessarily entail simple anatomical structure. The thallus is tough, like leather; it branches by forking (**bifurcation**) of its apex, which lies at the base of the **apical sinus** (or depression). If the shoot has forked shortly before, the centre of the previous depression is occupied by a lobe of the thallus, on both sides of which the apical depressions lie. Along the centre of each shoot projects, on its **ventral** [under] side, an indistinctly bounded midrib. From this

proceed outwards and forwards obliquely directed striæ arching towards the margin of the thallus. At some distance from the apex the thallus is fixed to the substratum by delicate rhizoids springing from its centre. If we bring the thallus, with its ventral side turned upwards, under a simple microscope, we can determine, by the aid of needles, the existence of **scales** which arise from the surface of the thallus. There are present here three different forms of ventral scales: **marginal scales**, which usually extend somewhat over the edge of the thallus, and have become brown; **median scales**, which lie in the middle line, and **laminar scales**, which are inserted upon the thallus on both sides of the middle line, can also be wanting. The median scales, often purple-coloured, alternate with one another, their edges overlap in the middle line. Together with median or laminar scales, or with the former only, arise out of the frond fine rhizoids, which, covered by the scales, and following their insertion, attain to the mid-rib, and here run further forwards in bundles. It is the median and laminar scales which produce the striation on the under side of the thallus, which we have already observed with the naked eye.

If we examine the **dorsal** [upper] side of the thallus with the lens, this appears to be divided into small diamond-shaped areas. The limits of the areas are dark-green, the areas themselves appear more grey. In the middle of each area a dot-like opening is visible. We now examine, with stronger magnification, a section which is taken parallel to the dorsal side of the thallus. We see that the outer cells of the dorsal surface are polygonal, firmly united together, and contain numerous large chlorophyll granules. The boundaries of the areas show clearly; each area has its centre occupied by a round opening, which is surrounded usually by four narrow cells containing no chlorophyll, and curved into the form of a crescent (Fig. 72, *A*). Where the section is somewhat thicker, air is seen to be collected under the free outer surface of the area. Into this air space, the **air-chamber**, project chlorophyll-containing threads of cells. The walls bounding the air-chamber laterally are constructed of closely-combined cells. These walls are uni- to multi-lamellar; their cells contain chlorophyll. Single cells of the surface, and also of the interior, are distinguished by a highly-refractive, irregularly-outlined, grape-like body. These bodies in the younger shoots are slightly brownish, in older are coloured brown, contain mostly fat oil, and form the so-called

oil-bodies of the Liverworts.[3] The cells which contain such a body show no other formative contents. Surface-sections taken from the ventral side of the thallus show no division into areas. The cells are here more elongated and poorer in chlorophyll than on the upper side. The rhizoids which spring from the ventral surface, show a double structure. They are more slender, and provided with peg-like projections into the interior, or thicker and without such thickenings. Those with the peg-like projections arise out of the frond as far as the median or laminar scales, or only the former, extend. They lie close to the frond, and follow the mid-rib in bundles, covered by the scales. They serve, perhaps, the purpose of stiffening the thallus.

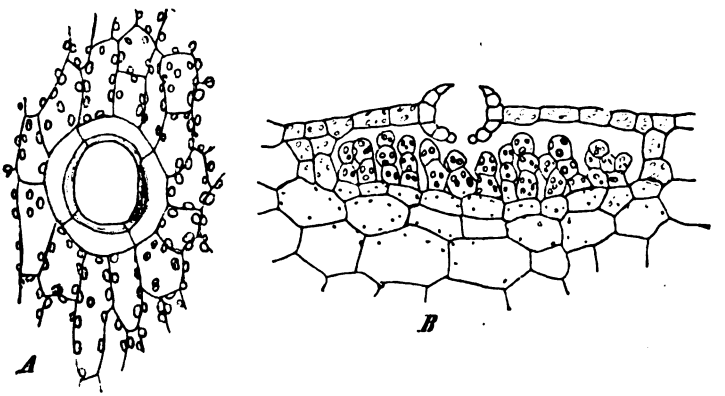


FIG. 72.—*Marchantia polymorpha*. *A*, an air-opening from above. *B*, in cross-section (× 240).

The slender rhizoids proceed chiefly from the midrib, and turn at an acute angle towards the substratum, to which they fix the thallus. At their apex they often appear sinuately lobed, at the base commonly purple-coloured. All ventral scales are unilamellar, the median consist of still living cells, the laminar and marginal scales of cells which quickly die.—A cross-section through the thallus shows us on the dorsal surface first a zone of chlorophyll-containing tissue. The interior of the thallus is composed of broader cells, almost free from chlorophyll. In the walls of these cells stellate broad elliptic pits are to be seen. At the ventral surface the two last layers of cells are again narrower, flatter, rich in chlorophyll, and form the so-called ventral cortical layer. Oil-bodies are scattered through the entire tissue. Other individual

cells are noticeable from their size and highly refractive contents; these are the **mucilage-cells**, which in *Marchantia* are feebly, but in other Marchantiaceæ are more strongly, represented.—A closer study of the outer layers, rich in chlorophyll, of the dorsal surface, completes the conception which we had obtained from the surface-view. Outermost we see a single layer of flat cells, which proceed from the walls bounding the air-chamber laterally, free over the chamber. In the midst of the free outer wall is found the **air-opening** [the so-called stoma], which is surrounded by several, from four to eight, tiers of cells[4] (Fig. 72, *B*). The opening is narrowed at its upper and under apertures, especially at this latter, and therefore shows with a barrel-shaped form. The cells of the uppermost stage are prolonged into a membranous border. As the air is very strongly retained in the air-opening, and the structure is thereby made indistinct, it is desirable previously to pump the air out of the preparation. Into the air-chamber project from below threads of cells, two or three cells long, and now and again branched. These threads are especially rich in chlorophyll; they arise from the flat cell-layer next below, which is poor in chlorophyll. On the ventral side of the thallus we see on the midrib the lateral, alternate overlapping of the median scales. Between the scales lie the cross-sections of the bundle of rhizoids. Median longitudinal sections show the insertion alike of the stronger ordinary rhizoids, turning off at once from the thallus, and the "pegged" rhizoids overlying the midrib.

A very simply constructed, and in many respects very instructive, thallus is that of *Metzgeria furcata*.[5] This inconspicuous plant is met with on the cortex of leafy trees [is cosmopolitan, and very variable]. The thallus is riband-like, bright-green, bifurcate, and traversed by a midrib visible even with the naked eye. Apart from this midrib, the thallus is unilamellar, as can be readily proved under the microscope. It consists of polygonal cells, richly provided with elongated chlorophyll-grains. The narrow midrib projects from the ventral [under] surface much more strongly than from the dorsal [upper] surface. It consists, passing from above downwards (as can be readily proved by focussing to different depths), of broad, slightly elongated, then of narrow, elongated, and finally again of broader cells. The two outer layers of cells contain chlorophyll, the inner, on the other hand, do not. At the growing-point arise, from the ventral surface of the midrib, some rather short club-shaped hairs, at their for-

ward end filled with highly refractive contents. From older parts of the midrib, and also from the marginal cells of the thallus, arise bristles, which, under favourable circumstances, can at their extremity give rise to a lobed suctorial disk, and then functionate as rhizoids. These bristles always stand at the hinder end of the cell (*i.e.*, most removed from the growing point), from which they are cut off by a curved partition wall, which does not pass through the entire height of the cell in question, but only cuts off a corner or angle from it. As the cross-section shows, the inner cells of the midrib are distinguished by somewhat more strongly

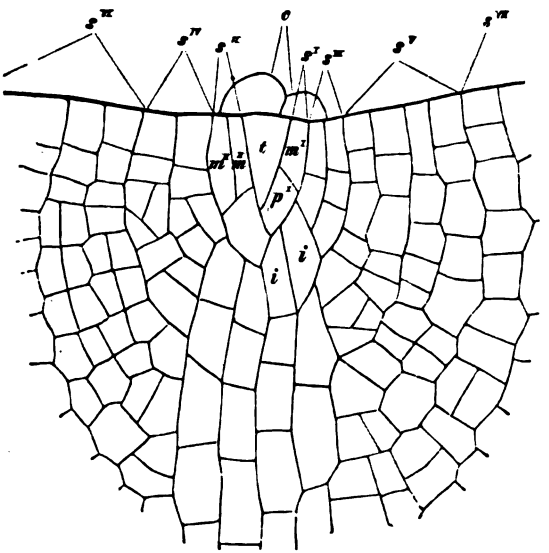


FIG. 73.—Apex of a shoot of *Metzgeria furcata*. *t*, Apical cell; s^{I}–s^{VII} successive segments; m_I marginal cell of the first, m_{II} of the second grade; p_I surface-cell of the first grade; *ii*, inner cells of the midrib; *c*, club-shaped hairs. The figure drawn by focussing into the inner cells of the midrib (×540).

thickened glancing white walls, appearing almost collenchymatous [*cf.* footnote to *Polytrichum*, p. 192]. In the most instructive and easy manner the processes of division in the growing point of *Metzgeria* can be followed.[6] The growing apex of Metzgeria shows a comparatively slight depression. The base of this **apical sinus**, close up to the place where the midrib commences, is occupied by the apical cell. We examine it from the dorsal surface of the thallus, in order not to be disturbed by the club-shaped hairs. The apical cell is wedge-shaped (Fig. 73, *t*). It

has the form of an isosceles triangle, with the base directed forwards (outwards), and usually somewhat convex, and slightly arched side-walls. It divides by walls which are parallel with its side-walls, and, thus developing, gives off segments right and left (*s*), and therefore all lying in one plane.

NOTES TO CHAPTER XVIII.

[1] Compare P. G. Lorentz, *Jahrb. f. wiss. Bot.* Bd. VI., 1867-8, p. 363; Goebel, *Grundriss der systematischen und speciellen Pflanzenmorphologie*, 1882, p. 184 (for the literature see also on p. 179); recently also G. Fritsche, *Ber. d. deutsch. bot. Gesell*, I. *Jahrg.* p. 83; *Haberlandt*, ditto, p. 263; and Oltmanns, in Cohn's *Beitr. zur Biol.* Bd. IV. p. 1.

[2] Compare Leitgeb, *Untersuch. üb. die Lebermoose*, VI. Heft, 1881, where the other literature is to be found.

[3] Pfeffer, *Die Oelkörper der Lebermoose*, *Flora*, 1874, No. 2.

[4] Voigt, *Beitrag zur vergl. Anat. der Marchantien*, *Bot. Zeit.* 1879. Col. 729.

[5] Compare Leitgeb, *Unters. üb. die Lebermoose*, Heft. III., p. 34, where also is the other literature.

[6] Compare Kny, *Jahrb. f. wiss. Bot.* Bd. IV., p. 85.

CHAPTER XIX.

VEGETATIVE STRUCTURE OF FUNGI, LICHENS, AND ALGÆ. STAINING THE CELL-CONTENTS.

Material Wanted.

Mushroom (*Agaricus campestris*). Fresh.
A Lichen, such as *Parmelia* (*Anaptychia*) *ciliaris*, common on trees. Fresh.
Fucus vesiculosus (Brown sea-weed). In alcohol.
Chara fragilis, or *Nitella*.* Fresh.
Cladophora glomerata.* A common fresh-water Alga. Fresh.
Spirogyra majuscula,* or other similar species. Fresh.

The vegetative organs of the **Fungi** consist, apart from a number of the simplest forms, of elongated, thread-like, more or less copiously branched elements, the **Hyphæ**. These are either without partition walls [**unseptate**], unicellular throughout their entire body; or, by means of partition walls [septa], segmented into a number of consecutive cells. Moreover, the most massive fungal structure is composed of such hyphæ, then very much interwoven with one another. The hyphæ can indeed, in many cases, become so firmly united, side by side, that a tissue is produced, which, as **pseudo-parenchyma**, delusively imitates the appearance of the parenchymatous tissue of the higher plants. The pseudo-parenchyma, however, is a product of the union of cell-threads, and not the result of cell-division taking place in three planes. In order to inform ourselves about this kind of structure, we take the fruiting body of a hymenomycetous fungus as subject for investigation. We choose the spore-producing body of the Mushroom *Agaricus campestris*, because the fungus can now be obtained at any season of the year, and shows, besides, a comparatively simple structure. We prepare first a delicate longitudinal section, from the stalk of a fully-developed specimen. We recognise clearly a structure of longitudinally disposed hyphæ, and can readily tear the section in longitudinal direction

* This, and many other Algæ, etc., can usually be obtained from T. Bolton, Newhall Street, Birmingham. [Ed.]

with the needles. The hyphæ are directed more or less parallel to one another; single ones run obliquely between the others. Each hypha forms a cell-thread, which is branched here and there by the formation of side-branches. These arise either close under a partition-wall, or else lower down on the side surface. Here and there we come across a "blind" end of a branch. The cells of neighbouring hyphæ not infrequently appear connected by a horizontal branch, and openly communicate with one another. In the periphery of the stalk the hyphæ are narrower, and at the same time more closely pressed together; just under the surface the walls become brown, and their cavities more or less completely collapse. Towards the middle of the stalk likewise the hyphæ become smaller, but their texture much looser, and, at the same time also, their course quite irregular; great masses of air here fill up the interspaces between the hyphæ. So long as the destructive influence of water has not made itself felt upon the contents of the hyphæ, very little of these contents is to be noticed; only at the cross-walls does it show, here and there, more markedly collected. Later on large **vacuoles** begin to form in the cells. Here and there small **crystals** are met with in the cells.

The cross-section of the stalk has a parenchymatous appearance, which is only lost in the middle parts of the section, where the hyphæ also offer their side views. This pseudo-parenchymatous tissue appears as if composed of unequal, irregular polygonal cells, which leave between them more or less numerous inter-cellular spaces and gaps (Fig. 74). On careful examination of the section, we notice close in the middle of many cells a refractive point (*cf.* the figure). The section has here grazed a cross-wall, and the middle point shows the position of a pit, which is clothed, on either side of the partition wall, with a small collection of a highly refractive substance. Such pits in the centre of the cross-wall are universally distributed amongst Basidiomycetes and Ascomycetes[2]. The cells of the hyphæ contain in the peripheral protoplasm, numerous very small **nuclei**, which, however, are not easy to see, and the method of identification of which we shall postpone.

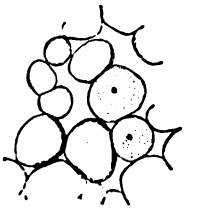


FIG. 74.—*Agaricus campestris*. Part of a cross-section through the stalk. In two hyphæ the section has grazed the cross-walls; a central point can be seen upon them (× 540).

Upon the structure of the stratum (thallus) of **Lichens**, we shall best obtain information by *Anaptychia* (*Parmelia*) *ciliaris*, universally distributed on tree stems. The thallus itself is erect, leaf-like, and shrubby [foliaceous-fruticose]; on the dorsal [upper] surface, grey-green to bright green; on the ventral [under] surface grey. From the edges of the thallus arise stiff **cilia**, which often become lobed at their ends, and, where they extend to the substratum, adhere to it. We hold a piece of the thallus between two pieces of elder-pith, and cut cross-sections through it. By a sufficiently strong magnification, we see that the thallus consists, on its dorsal surface, of closely interwoven, thick-walled hyphæ. These form the so-called rind, or cortical layer. Passing further inwards, the curves of the hyphæ separate from one another, in order to form the looser central layer. We can readily decide that the hyphæ are long sacs, branched from time to time, and divided by cross-walls. At the limits of rind and central tissue lie scattered comparatively large, green, globular cells, the **Gonidia**. They correspond with the alga *Cystococcus humicola* [= *Chlorococcum humicolum*]. The hyphæ fit closely to the gonidia, and carry to them the crude sap, for which they receive in return a portion of the substances assimilated in the gonidia. There exists here a **symbiosis**, a conjoint existence of fungus and alga, which is based upon reciprocal service. At the under surface of the thallus of *Anaptychia* the fungal hyphæ again interlace more closely, so as to form a kind of under rind; or this closer combination does not exist, and the looser central tissue extends to the ventral surface. This latter is in general the case. At the edges of the thallus, however, the rind of the dorsal surface, in all cases, extends underneath to the ventral side. From these edges arise, as we have already determined macroscopically, the fixing cilia (**rhizines**), which now can be made out to consist of parallel closely-combined hyphæ. The walls of these hyphæ have a brownish colour. At their base the threads often fork. In other Lichens the rhizines are apt to spring mostly from the ventral surface of the thallus. Chlorzinc iodine stains the walls of the gonidia immediately a beautiful blue, while the hyphæ only appear yellow to yellow-brown, showing the reactions of the so-called **fungal cellulose**.

In *Anaptychia ciliaris* we have a Lichen with what is called a layered or **heteromerous thallus**, so called because the gonidia form a special layer in the thallus. In more lowly organized

lichens the thallus is **homoiomerous**, *i.e.*, the gonidia are distributed through the whole tissue. To the last belong also the **Gelatinous lichens**, in which the gonidia lie in a translucent jelly, which is penetrated by the hyphæ of the fungus. Moreover, the Algæ, which take part in the formation of the lichen-thallus, differ according to their species, are coloured green or blue-green, but belong almost exclusively to the lowest divisions of the Algæ.

The thallus of the Algæ shows a wide structural range of variation; from a very simple to relatively complicated structure. As an example of the latter, we will take the olive-green or brown seaweed, *Fucus vesiculosus*, known as the "bladder wrack," and which is so common around our coasts. This *Fucus* is, in its younger parts, flat, leaf-like, traversed by a **midrib**, which projects on both sides, and forks more or less regularly in the plane of the leaf-like expansion. It usually bears bladdery swellings, often situated in pairs one on either side of the midrib as well as singly at the base of the bifurcations; these bladders are sometimes wanting. In the older parts the leaf-like expansion of the thallus is gradually disorganised, and finally is reduced to the considerably thickened midrib, which in section has become elliptic, and suggests a stem. The stem ends below with a rounded attaching disk. Several stems may arise from a common base. From the older parts of the thallus, especially from its edges, numerous **adventitious shoots** can often arise. The **growing points** of the thallus lie at the apices of the twigs, in cleft-like depressions, the direction of which corresponds with the plane of the leaf-like expansion, and which can be readily recognised with the lens. Some of the apices may be seen to be double, from equal forking, or dichotomy, but quickly one fork surpasses the other, the latter becomes pressed to one side, and appears as if laterally developed. For anatomical investigation alcohol material is almost as well suited as when fresh; but fresh material can be sent for great distances without injury, when packed in cases without water. In what follows, therefore, we will concern ourselves preferably with alcohol material, as this permits section-cutting much more readily. In fresh material the swelling of the tissue between the outer and inner layers of the thallus is so strong that it produces great contortion of the sections. The outer layer is in **positive**, the inner in **negative tension**; that is, the outer layers are compressed by the inner layers, and the latter stretched by the outer. Therefore in the sections the outer layers lengthen and the inner layers shorten, and the sections twist. Prepara-

tions made from fresh material must be examined in sea-water, since they swell strongly in fresh-water; alcohol material, on the other hand, we lay a few days previously in a mixture of equal parts alcohol and glycerine, and examine it in the same. We prepare surface sections, parallel to the surface of the thallus, through the midrib and the wings, (and, if we wish to see only the primary tissue, at not too great a distance from the growing point,) besides cross and longitudinal sections through different regions. The entire surface of the frond shows, in surface sections, rectangular to polygonal cells, which are arranged more or less clearly in longitudinal rows, often displaced by supplementary divisions. In cross and longitudinal sections these cells appear prismatic, and elongated perpendicularly to the surface. They are clearly distinguishable from the layer of tissue which lies next below, and we will distinguish them as the outer or **epidermoid** layer. The cells of this outer layer are closely filled with olive-coloured **chromatophores.** The chromatophores have the form of rounded grains, or are become polygonal by lateral pressure, and contain the characteristic colouring matter of the Fucaceæ, **phæophyll.** To the outer layer succeed elements with wide cavities, which in cross-section appear approximately polygonal, but, as the longitudinal section shows, gradually increase in length as we pass further into the thallus. They contain larger chromatophores, which however are not so crowded as in the outer layer, and therefore can be more readily observed. We include these cells, together with the outer layer, as cortex. The cortical cells are connected together by pits with a porous closing membrane. The innermost layer of the cortical cells bears to the midrib the character of a **thickening layer,** which commences its activity at some little distance from the growing point.—By surface-sections below the cortex, and by suitable longitudinal sections, we determine that the interior of the midrib consists of a tissue of longitudinally elongated cells, running parallel to one another and to the long axis of the frond, and which are connected together into threads. Laterally, these cells communicate by broad pits, and in part also by short prolongations. In the longitudinal direction, the cells of the threads are only separated by delicate partition walls, which are clearly interrupted in a sieve-like fashion. A similar structure is shown by the partition walls inside the lateral prolongations, and by the closing membranes of the pits. The threads are separated laterally by an intercellular jelly, which has arisen from the swelling of the

middle lamellæ and outer thickening layers of the longitudinal walls of the cells. The "pith" of the midrib passes over laterally into the "packing tissue" of the two wings, and this appears as a looser network of irregularly disposed cells, constructed much as in the pith. Here also the partition walls in the cell-threads and the lateral connections are thin and with sieve-like interruptions, while the longitudinal walls have produced a still more abundant jelly than in the midrib. The contents of the cells of the pith and of the packing tissue are poor in chromatophores, but on the other hand are often rich in highly refractive granules, which cannot be removed by alcohol, but can by ether, which become brown in osmic acid, and therefore are recognised as fat oil. In each cell a nucleus can be made out. Not infrequently the protoplasmic cell-contents take on a chambered structure in the water in which we investigate them. With the addition of iodine, the cell-contents, excluding the oil-drops, colour yellow-brown, the nuclei are usually easily visible; a starch reaction is found nowhere, and the oil here no doubt replaces the missing starch. The whole distribution of the tissues leads to the conclusion that the processes of assimilation are localised in the cells of the cortex, while the cells of the pith and the packing tissue furnish the conducting system. If we treat the sections with chlorzinc-iodine, or with iodine and sulphuric acid, we obtain in either case, but especially with the latter, a blue coloration of the walls. The firmer membrane immediately surrounding the cell-cavity colours deeply, less deeply the more distant; in the lowest tissue, filling the wings of the thallus, the coloration of the jelly tends to be entirely lost. The parts of the membrane around the cell-cavities show distinct lamination. If we allow hæmatoxylin solution to act upon the section, the layers around the cell-cavities stain an intense deep violet, the whole jelly takes a bright violet tone and becomes everywhere readily visible.

Even with the naked eye we observed that the thallus in its older parts is reduced to a midrib swollen into a strong stem. The growth in thickness which brings about the development of this stem out of a midrib takes place in the innermost layers of the cortex. The cells of these layers develop from their lower end sac-like prolongations, which, dividing by cross-walls, and branching from time to time, grow downwards in the jelly between the threads of the pith. Only here and there one of these thickening threads grows into the packing tissue of the wings. Longi-

tudinal sections through the neighbourhood where noticeable increase of thickness of the midrib begins, shows us these phenomena without any difficulty. If we examine the cross-section of the deeper parts of the stem, we find it constructed internally of sparsely scattered cells, with wide cavity and brownish contents, and in between these by numerous closely pressed cells, with narrow cavity and greenish contents. The former are the original threads of the pith, which serve for food conduction; the latter are those which have been intruded in growth in thickness, and have to sustain mechanical functions. The threads originally present have been pressed apart from one another by those formed later. The base of the stem and the attaching disk are, in fully developed specimens, exclusively composed of these mechanical threads. In the outer part of the stem the tissue has also undergone a change. The cells of the outer layer have become brown, are dead, and are gradually cast off. The second layer of the cortex has begun to divide by periclinal walls. We find therefore in the exterior parts of the stem radially arranged rows of cells, and this, in stronger stems, to a not inconsiderable degree. The wings of the thallus gradually die up to the midrib, while the growing cortical layer in the periphery of the stem gradually closes together.

If the thallus is held up to the light and observed with a lens, we notice a number of, as a rule, irregularly scattered dots, which are wanting only over the veins and midribs. Even with the naked eye these dots appear as protuberances. On surface sections each appears as a round opening, surrounded by a projecting rim, and out of which a tuft of long hairs projects. They are pits, no doubt allied to the conceptacles which we shall study hereafter in considering the sexual processes, and which we will call now **sterile conceptacles**. If we prepare a cross or longitudinal section through such, it appears as a flask-shaped hollow. The hollow is surrounded by the cells of the inner cortex. From the cells at the base of the hollow arise long threads, composed of elongated cells, which project from the mouth of the sterile conceptacle. It is quite possible that these hairs facilitate the absorption of nutrient materials from the surrounding water. In sections through older parts of the thallus, we can find, between the long hairs in the pits, also bundles of shorter-celled hairs, which do not reach to the mouth of the pit. If, lastly, still older parts are examined, where the pits present themselves as brown

spots, we find the outer parts of the long hairs destroyed, and the opening of the pits closed by the basal parts of these hairs, by the short hairs, and by a brownish mucilage.

Sections which are taken through a young bladder of the thallus, show its interior filled with a plexus of the same threads which we found in the wings of the thallus. Scattered in the jelly between the threads are bubbles of gas, which sometimes tear the loose tissue and form large air-chambers. The older bladders are quite hollow, filled with air, remnants of the threads of cells being found on the wall. This latter is covered by the outer layer, and shows a thick cortex, arisen from the tangential division of the other cortical cells.

The small but widely distributed family of the **Characeæ** (or Stoneworts) occupies a well-nigh isolated position in the Vegetable System, but can best be included amongst the green Algæ. They are characterised by a remarkable vegetative structure, and, as we shall see hereafter, by a still more remarkable structure of the sexual organs. They are not at all uncommonly found growing at the bottom of ponds and streams, and in bog holes, forming long dense tufts of green thread-like stems, rooted in the mud by means of delicate hairs, or **rhizoids.**—The stoneworts are divided into two genera, *Nitella* and *Chara*, distinguished for our present purpose by differences in the structure of the stem. As the more simply organised, we may commence our study with *Nitella*. The slender axis examined with the naked eye is seen to be segmented, long bare segments alternating with whorls of appendages. The former are the **internodes**, the latter arise from the **nodes**. We have here therefore an apparent approach to the condition of affairs met with in the flowering plants already studied. Each internode consists of a single very elongated cylindrical cell. The principal characteristics of this **internodal cell** we have already examined (see p. 37). It possesses a firm, well-developed, transparent cell-wall; underlying this is a lining layer of protoplasm, in which are embedded innumerable regularly arranged chlorophyll-bodies, with thin layers of protoplasm between them. Within the stationary layer is a layer of protoplasm in the active streaming movement known as **rotation**. The general cavity of the cell is filled with cell-sap. The **neutral lines** will be readily visible;

these, and the lines of chlorophyll-bodies pass spirally along the cell. The node consists of a single disk-like cell, on the edges of which are borne appendages ("leaves," or "branches"), likewise consisting each of a thread of cells. From the lowermost nodes arise long, often branched rhizoids, divided by oblique partition walls. As we pass higher up the stem the internodes become progressively shorter, and the nodes and their appendages therefore nearer together, until at the summit they are crowded together into a terminal **bud.** Dissect out this bud under the microscope by means of needles. This is best done from materials laid for from 12 to 24 hours in a 1 p.c. watery solution of chromic acid. The bud is laid upon a slide, and with needles the lowest portions are successively removed so long as it appears safe to do so. These portions are then removed out of the way, a small drop of glycerine added to the specimen, the cover-glass laid on, and by gentle pressure with the needles upon the cover-glass, while observing through the microscope, the bud slightly crushed. It is probable that the bud will open, and expose its apical structure. At the apex is seen a single hemispherical cell, the **apical cell,** in which is visible a well-defined protoplasmic body, and a rather irregularly shaped nucleus. In some cases two nuclei will be seen, placed one nearer the flat side of the cell, and the other nearer the free apex; such a cell is about to divide. The dividing wall is formed parallel to the flat wall of the apical cell. In this way cells are cut off from the apical cell. The cell which resulted from the previous division will be seen adjoining the flat wall. Tracing back from the apical cell, it will be seen that the cells become alternately nodal and internodal cells.

Let us now turn to *Chara*, selecting for the purpose the widely spread *C. fragilis.* Externally this plant resembles *Nitella*, but it will be seen upon examination that the internodal cells, instead of being naked, are covered with a layer of cells which we call the **cortex,** consisting of slender cells which are themselves divided into cell-rows. Similarly, the nodes are flat disks of cells, of which the external ones give rise to the whorls of appendages and also to the cortical cells. Rhizoids arise from the lower nodes, as in *Nitella.* The leaves are segmented like the stem, and from their lower nodes produce leaflets. The terminal cell of the

leaf has no cortex, and in it the general features of the internodal cell of *Nitella* are repeated. The bud resembles that of *Nitella*, excepting that the nodal cells undergo early segmentation by vertical walls, so as to produce a disk consisting of a central and a layer of external cells, these latter growing out in such fashion as to overlap the undivided internodal cells, and, by subsequent division, produce the cortex.

The Cladophoreæ[3] present themselves to us as abundantly branched green threads, whose segments decrease in thickness with the grade of the branching. They are the most widely distributed of all fresh-water algæ, and any species is suited for examination. The determination of species is, however, very uncertain in this genus. We select a dark-green, undulating, tuft-forming *Cladophora glomerata* for further examination. This is corymbosely branched, the side-shoots arising, as in all other Cladophoreæ, from the upper end of the constituent cells. The branching proceeds acropetally, so that the end cells of the branches act as apical cells. Subsequently branches arise also from the older segments, producing what we may call **adventitious shoots**. With sufficiently strong magnification, the green peripheral protoplasmic layer of the cells shows to be composed of small polygonal plates, the **chromatophores** (Fig. 75, *ch*), separated by delicate colourless lines. In each plate more or less numerous pale grains (of starch) can be seen; besides these, in some plates, lie comparatively large, more or less regularly globular bodies, more strongly refractive, which are generally known as **amylum-bodies**, and more recently as **pyrenoids**[4] (*p*), and in which an inner grain is more or less clearly distinguishable from an outer layer. The cells are seen to be filled internally with

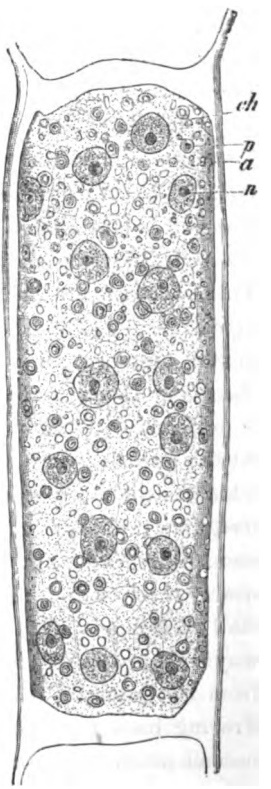


FIG. 75.— *Cladophora glomerata*. A cell of a thread from a chromic-carmine preparation. *n*, a nucleus; *ch*, chromatophores (colour-bodies); *p*, amylum-bodies (pyrenoids); *a*, starch grains (× 540).

cell-sap, which is traversed by colourless, exceedingly thin **protoplasmic plates**, which, proceeding from the peripheral layer, divide the cell cavity into irregular, unequal, polygonal chambers. Here and there **chromatophores** [colour-bodies] can be seen in the inner protoplasmic plates. By focussing so as to get an optical section, it will be seen that colourless protoplasmic balls here and there project from the peripheral protoplasm into the cavity of the cell. These are the **nuclei**, in which, in especially favourable situations, a **nucleolus** can also be distinguished. In *Cladophora*, as is clearly proved by this examination, we have to do with **multinuclear cells**. If now the preparation is somewhat firmly crushed, we see in the flattened cells, the contents of which are somewhat withdrawn from the walls, the individual chlorophyll-plates, separated from one another, and rounded. At the same time the small grains and amylum-bodies show up clearly in the chromatophores, which now appear, like the chlorophyll-grains of higher plants, acted upon by the water. If we now add a little potassium-iodide-iodine solution to the preparation, the small grains, and also the outer layer of the amylum-bodies, colour violet; in the green chromatophores, however, they appear brown, and the partially visible nuclei also take a brown colour. We must not omit in this preparation to look out uninjured cells, in which starch-grains and amylum-bodies are stained in their natural position, and are very well defined, and we can also, by deeper focussing, distinguish the nuclei. We now examine another thread, which we lay directly into a drop of picric-alcohol, when the nuclei of the amylum-bodies are sharply defined in the yellowish-brown stained protoplasm. Sufficiently strong magnification presupposed, these bodies appear angular; they are **protein crystals**,[5] of which, moreover, two not infrequently lie in an amylum-body. After a short time irregular brown bodies appear in the chlorophyll-plates, which proceed from the disorganized chlorophyll, and give us the **hypochlorin** or **chlorophyllan** reaction[6]. The same reaction will be obtained under the influence of other acids. However, in order to be able to study the nucleus more closely, and to obtain a complete insight into their distribution, we will bring other methods into use. This will besides give us opportunity of learning some approved methods of "fixing" and staining, which histological studies have, in recent times, to thank for not unimportant advances. We place some branches of the *Cladophora* in 1 per cent. chromic acid, other small portions in con-

centrated picric acid, still others in 1 per cent. chrom-acetic acid (chromic acid 0·7 per cent., acetic acid 0·3 per cent.)[7]. In doing this, we must take care that the reagent is at least 100 times the bulk of the object to be fixed. The 1 per cent. chromic acid, and the chrom-acetic acid, we allow to act for some hours, even without disadvantage for 24 hours, and the picric acid for about 24 hours. All these objects must afterwards be washed most carefully in distilled water; they can with advantage remain for up to 24 hours in water which is frequently changed. Specially careful treatment is required by the picric-acid preparations when they have to be stained with hæmatin-ammonia.—The variously "fixed" and well-washed preparations are now laid in watch glasses with Beale's carmine,[8] with Thiersch's or Grenacher's borax-carmine, and also with Hoyer's neutral carminic-ammonia. In Beale's carmine the sections must remain for up to 24 hours, about half the time in Hoyer's carmine, several hours in borax-carmine. Another portion of the threads we stain with Grenacher's or Boehmer's hæmatoxylin [logwood], which, if it is to stain well, must be as old as possible. This solution is used very greatly diluted. It is best, from time to time, to control the extent of the staining of the object by slight examination under the microscope, and to take it out when it has taken up sufficient colour material.* If, in spite of this care, the object should be overstained, that is should be stained too darkly, it is laid in pure water, or in watery alum solution, or in water containing a trace of hydrochloric acid, and left in the fluid in question until the intensity of the coloration is diminished to the required degree. If the preparation has been treated with acidulated water, it is necessary afterwards to wash the preparation for some minutes in very weak ammonia water. In order to be able to stain the preparation according to the hæmatin-ammonia method,[9] we must have previously removed from it every trace of picric acid. For this purpose we transfer it to a comparatively large quantity of boiled water, which we repeatedly change. In this water, freed from carbonic acid gas by its previous boiling, the object remains for from 24 to 48 hours, after which it can be stained. For this purpose we throw some crystals of hæmatoxylin in a small quantity of distilled water,

* This is perhaps best effected if, instead of staining in a watch-glass, the process is carried on upon an object-slide, in which a round or oval hollow has been ground. The slide can be placed bodily on the stage of the microscope, and is handier in use for this than a watch-glass. [Ed.]

and aërate it with ammonia gas. This latter we effect with the aid of a wash-bottle containing some ammonia solution, in which the two glass tubes do not reach the fluid. The hæmatoxylin now dissolves with a beautiful violet colour. The solution is greatly diluted with distilled water, and the preparation allowed to lie in it for about two hours. The exact time for coloration can here also be directly controlled. The preparation is, with advantage, somewhat overstained, and afterwards steeped for several hours in distilled water. This method of staining is somewhat troublesome, but often gives, however, the most exquisite results. Preparations hardened otherwise than with picric acid, are little suited for staining with hæmatin-ammonia. The preparations treated with Beale's carmine, with borax-carmine, or with Hoyer's carmine, are likewise most beautiful when they are overstained, and afterwards laid for some time in a watch-glass, in 50 per cent. to 70 per cent. alcohol, to which is added a drop of hydrochloric acid. For this purpose we can keep ready prepared a solution of about $\frac{1}{2}$ per cent. hydrochloric acid in 70 per cent. alcohol. Previously these preparations show a more or less diffused coloration; they first acquire a definite staining in the hydrochloric-alcohol. The preparations laid in acidulated alcohol are in all cases washed afterwards with alcohol containing no acid.

If after completed examination we wish to make permanent preparations of the stained objects, we choose for our preserving medium either glycerine or glycerine-jelly, or, for carmine preparations, Hoyer's mounting fluid. If the hæmatoxylin stain is to be preserved in glycerine or glycerine-jelly, this must be completely free from acids. The foregoing preparations must not be transferred immediately to the enclosing medium in question, as otherwise the cells, as the result of sudden withdrawal of water, would collapse. These preparations are, therefore, first laid in very dilute glycerine, which, by standing exposed to air, very slowly concentrates. The threads can then, without prejudicial results, be transferred to glycerine, or to glycerine-jelly. The glycerine preparations are closed with Canada balsam. The glycerine-jelly, or Hoyer's mounting fluid, needs, as we have already seen, no further enclosing.

We will now submit the various preparations to close study, and find that the chromic acid, or chromic acid mixture, preparations, stained with forms of carmine on the one hand, and preparations which are suitably fixed and stained with hæmatoxylin

and hæmatin-ammonia on the other hand, show themselves in the foregoing cases to be the best. It must, however, be explicitly stated that this result is limited only to the objects in question, and for other objects another method, which here is less advantageous, might have the preference. It also happens only too frequently that a stain formerly approved fails for unknown reasons, and, therefore, a conclusion should never be based upon an isolated case. In general, the fixing and staining of the cell-contents has become a special art, which must be learnt, and requires practice, so that in our first attempts we must be prepared for failures. We have chosen *Cladophora* as a suitable object for introduction to the various methods of hardening and staining; whoever wishes to limit himself to the most certain, rarely failing, method, will harden in the above way in 1 per cent. chromic acid, and afterwards stain, one part with borax-carmine, another with hematoxylin. The borax-carmine stain almost always succeeds.

In the borax-carmine preparation (Fig. 74), the nuclei stand out quite sharply. The amylum-bodies (pyrenoids), together with the rest of the protoplasm, remain as good as unstained, and the starch-grains also take no coloration. The amylum-bodies now show clearly in their interior the more strongly refractive protein crystal, which is surrounded by a hollow ball, which gave us earlier the starch reaction with iodine. The nuclei, to which we specially turn our attention, are distributed pretty uniformly in the cell; they lie on the inner side of the chlorophyll-layer, and project into the cavity of the cell. Each nucleus shows a more darkly-stained nucleolus, and appears, besides, as if finely granular or finely porous. The hæmatoxylin or hæmatin preparations show the nuclei stained dark, and besides, though more faintly, the crystal in the chlorophyll-vesicle. The starch-grains are not stained, but on the other hand the **microsomata** (microsomes) of the cell protoplasm are, and almost as darkly as the crystals of the chlorophyll-vesicles (amylum-bodies).

The genus *Spirogyra* furnishes us with a simple filament or thread of cells. We choose for examination a species which has a central, readily-visible nucleus. So constituted, for example, is *Spirogyra majuscula*[10] [*S. orthospira*], which is met with now and then, not exactly rarely, but sporadically, in pools. For this purpose other species with central nucleus will serve equally well for examination, and will differ but slightly in the essential relations of their structure. If once in possession of good

Spirogyra material, you should endeavour to preserve it in cultivation. This is effected best in comparatively shallow vessels, whose walls are either opaque, or are made opaque by means of black paper, as light falling unilaterally acts disadvantageously. The vessels must stand in a light place, but protected from the direct action of the sun. Into either river or spring water, which is not too rich in chalk [too "hard"], are thrown from time to time boiled pieces of turf soaked in a nutrient fluid. This nutrient fluid will be prepared suitably if we add to 1000 ccm. water, 1 gram nitrate of potash, ½-gram sodium chloride, ½-gram sulphate of lime, ½-gram sulphate of magnesia, ½-gram finely pulverized phosphate of lime, and to it add a few drops of ferric chloride solution.[11] Under such circumstances the *Spirogyra*, and fresh-water algæ in general, thrive well. The cells of *Spirogyra majuscula*, when fully developed, are about 1½ to twice as long as thick (Fig. 76).

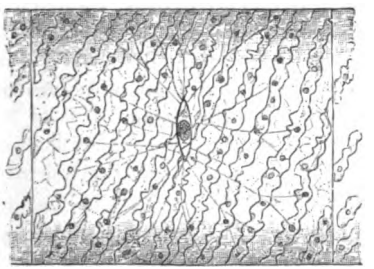


FIG. 76.—*Spirogyra majuscula*.* A cell of a thread gradually focussed into, showing therefore, besides the chlorophyll-bands, the nucleus with its suspending threads (× 240).

The cell-wall is lined by a delicate, colourless, peripheral layer of protoplasm, which becomes clearly visible if the cells are **plasmolysed**, *i.e.*, if the protoplasmic body of the cell is made to contract by some water-withdrawing medium, such as sugar-solution, glycerine, solution of common salt, or of salt-petre. To the colourless lining-layer follow 8 to 10 **chlorophyll-bands**, which usually appear pretty steep and closely wound. The bands have a finely undulating outline, and are transparent enough to admit of a view into the interior of the cell. At irregular distances in the bands are imbedded denser, globular, colourless, bodies—the amylum-bodies with which we are already acquainted. The amylum-bodies show a protein-crystal, and a hollow globe of small starch-grains as an enclosing sheath. We recognise the angular outline of the crystals even without reagents; they stand out more sharply if some picric alcohol is run under the cover-glass. By treatment with potassium-iodide-iodine, from the

* The synonymy of the *Spirogyra majuscula* of the text is rather obscure. This figure does not resemble *S. majuscula* of Kutz. = *S. orthospira*, of Näg. and Archer. The most common of the thick-threaded Spirogyras in Britain is *S. nitida*. The figure more resembles *S. orbicularis* of Hassall. [ED.]

conjoint staining of the starch-sheath and the protein crystal, the whole body appears dark-brown. Chlorzinc iodine acts very effectively, making the starch-grains swell to a cloudy blue film, through which the crystal shows clearly. The central nucleus in this species is spindle-shaped; it becomes, nevertheless, by pressure upon the cell, brought out of its position and visible from its side, and then presents the form of a disk; it has, therefore, in reality the form of a bi-convex lens. In its centre lies a large distinct **nucleolus**; seldom two or three such bodies are distributed symmetrically in the interior of the nucleus. In other more nearly-allied species the nucleus is thicker, and appears in its natural position in the cell as rectangular, with rounded corners. The nucleus is surrounded by a very thin layer of protoplasm, from which delicate protoplasmic threads run out towards the peripheral protoplasm of the cell. By these threads the nucleus is suspended in the cell-sap filling the cavity of the cell. The threads all arise from the thin margin of the nucleus, usually fork repeatedly in their course, and join on to the inner side of the chlorophyll bands, and in all cases at the projecting parts which cover the chlorophyll-vesicles (pyrenoids). We can convince ourselves of this in most cases easily by slowly changing the focus.

NOTES TO CHAPTER XIX.

[1] H. Hoffmann, *Icones anal. fung.*, I.–III.; De Bary, *Morph. d. Pilze*, etc., pp. 49 et seq.

[2] On the pits in the partition walls of the Florideæ, compare Bornet, *Etudes phycologiques*, p. 100; and Schmitz, *Stzber. d. kgl. Akad. d. Wiss. z. Berl*, 1883, p. 218.

[3] Schmitz, *Siphonocladiaceen*, p. 17; Strasburger, *Zellbild. u. Zellth.*, III. Aufl., p. 204.

[4] Schmitz, *Chromatophoren d. Algen*, p. 37; compare also pp. 16 and 35.

[5] According to a communication from A. W. Schimper.

[6] Pringsheim, especially in the *Jahrb. für wiss. Bot.*, Bd. XII., p. 294; A. Tschirsch, *Ber. der deut. bot. Gesell.*, Bd. I., p. 140, where the literature is also given.

[7] Flemming, in *Kernsubstanz, Kern- und Zelltheilung*, 1882, p. 379, where the literature also is given.

[8] The property of the nucleus to take up and accumulate colouring matters was discovered by Th. Hartig: "Ueber das Verfahren bei Behandlung des Zellkerns mit Farbstoffen," *Bot. Zeit.*, 1854, col. 877. *Entwicklungsgesch. d. Pflkeims*, 1858, p. 154. In animal histology the treatment was introduced by Gerlach. *Mikr. Stud. a. d. Geb. d. menschl. Morpholg.*, 1858.

[9] Compare Schmitz, *Stzbr. d. niederrh. Gesellsch.*, 13th July, 1880; separate reprint, p. 2.

[10] Strasburger, *Zellbildung und Zelltheilung*, III. Aufl., p. 173.

[11] Nutrient fluid, according to Sachs, *Vorlesungen über Pflanzen-Physiologie* p. 342.

CHAPTER XX.

DIATOMACEÆ, PROTOCOCCUS, YEAST, SCHIZOPHYCEÆ, (SPLITTING ALGÆ).

Material Wanted.

Some large diatom, *e.g.*, *Pinnularia* (*Navicula*) *viridis*. Living.
Protococcus viridis, from a tree trunk. Living.
Hæmatococcus (*Protococcus*) *pluvialis* from a water-butt or roof-gutter. Living.
Yeast (*Saccharomyces cerevisiæ*), from a brewery. Living.
Anabœna Azollæ, or *Azolla caroliniana*. Living.
Oscillaria, sp., from standing water or wet soil. Living.
Gleocapsa, sp., from damp walls, or the glass of a fernery. Living.

The Diatomaceæ or Bacillariaceæ are **unicellular** organisms, occupying an intermediate position between animals and plants, and form an isolated group. The most favourable object upon which to get information as to the structure of the Diatomaceæ is, perhaps, *Pinnularia* [*Navicula*] *viridis*,[1] a species very common in standing and flowing fresh water. It is distinguished amongst fresh-water forms by its comparatively large size, and allows in general an easy insight into the structural relations of its body. Under the microscope, in which we must study them with the strongest objective at our command, they appear either in the form of an elongated ellipse or as a rectangle with somewhat rounded ends. In the former case, we see them from the side of the **valve** [**frustule**] (Fig. 77, *A*), in the latter, of the **girdle** [or joint of the valves] (Fig. 77, *B*). We will call these the **valve-side** and **girdle-side** respectively. On the valve-side, the cell-wall appears marked with narrow furrows, running from the edges towards, but without reaching, the middle (compare the figure). They are usually considered to be depressions in the outer surface of the valve, *i.e.*, thin places therein. The central, smooth space, free from the furrows, shows at its middle and each end, a strongly refractive thickening, which we distinguish as a **nodule**. The two end nodules are joined to the median nodule by a line, which bends out symmetrically close on either side of the median nodule, and

ends in a slight enlargement. The end nodules are surrounded by the ends of the line in the form of a crescent, to permit which the lines bend out at both ends laterally in the same direction as at the median nodule. In its course between the nodules, the line broadens a little. We assume that it is a cleft leading into the interior of the cell; it is the **raphe**. The furrows do not pass on to the girdle side (*B*); we see them only at the edges of the figure. By focussing for the optical section, and careful examination of the ends of the cell, we can demonstrate the remarkable fact that the middle line of the wall is double. From exhaustive investigation, it is settled that there is here an overlapping, box-wise, of the separate parts of the wall. At the edges of the two elliptic wall-segments, which we saw in the view of the valve-side, portions of a membrane adjoin, which end with free margins. The wall of this cell, therefore, consists of two halves, of which the one is inserted inside the other. The structure of this wall indicates throughout that of an elliptic box with a cover placed upon it. The side walls of the cover are just as high (deep) as those of the box, but they are not completely slipped the one into the other. If we return, in our cell, from the optical section to the surface view, we can follow the thin edges of the two halves of the cell as delicate lines. The grooved surfaces of the cell-wall we distinguished as **valves**, the smooth free-ending side walls as **girdles**, whence the use of the terms in question to indicate the two views. In *Pinnularia* it is easy to free the one half of the cell-wall from the other by pressure or by chemical reagents, and, moreover, here and there dead specimens are found in which this process has more or less completely taken place. With pressure the girdles easily break at some little distance from their edge, and along a line parallel with it. These lines, one near each edge, and therefore two in girdle-view, are often recognisable, and may be thin parts of the girdle. They do not extend to the ends of the cell. The contents of the cell present a somewhat different appearance according to whether we have a valve- or girdle-view. In the former (Fig. 77, *A*), a median clear strip traverses the cell from end to end; the colourless **cytoplasm** of the cell is therefore visible. In the mid-length of the cell it appears collected into a bi-concave protoplasmic bridge. In this "bridge" lies the **nucleus**, not always readily visible without the use of reagents, and with a comparatively large **nucleolus**. Bounding both sides of this clear band, with a tolerably smooth or undulating outline, are the brown-

coloured chromatophores (colour-bodies), the **endochrome plates.** These lie, therefore, on the sides of the girdle. In the protoplasmic "bridge" can be seen narrow rodlets, connected in pairs, the meaning of which is unknown. Lastly, in the **cell-sap** lie usually, but not always, larger and smaller **oil-drops.** In the girdle-view the cell-body appears uniformly brown, because here the chromatophore covers the whole colourless peripheral protoplasmic layer. Only at the two extreme ends of the cell does the colourless protoplasm come to view. The chromatophore is uniformly dense and uniformly coloured, without visible differentiation. In girdle-view also the central collection of protoplasm appears to have the form of a bi-concave bridge.

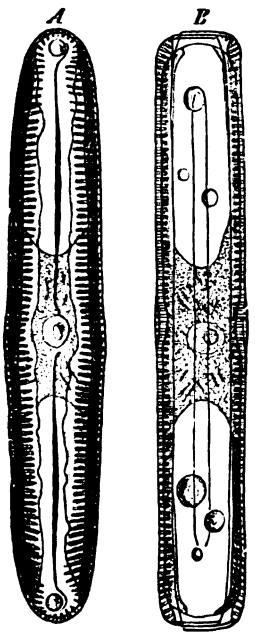


FIG. 77. — *Pinnularia viridis.* *A*, View of the valve-side. *B*, View of the girdle-side (× 540).

If we examine now our former preparation of *Cladophora*, we are pretty certain to find diatoms clinging to this alga. They were fixed and stained at the same time with the alga, and we shall see the stained nucleus beautifully in each cell.

Amongst a large number of examples of *Pinnularia* we may here and there find one double. These are **sister-cells,** which have recently resulted from the division of a **mother-cell.** They cling to one another with their valve sides, and, if the wall is fully developed, we can determine that the girdles of the two inner valves are inserted in the two outer valves. After division of the contents of the mother-cell, these inner halves of the wall are developed for each individual. Each cell, therefore, has an older and a younger half of its wall *i.e.*, one valve, the outer one, belonged to the mother-cell, and the other, inner valve, is peculiar to the present individual, and this consideration shows that the difference of age between the two valves may be very considerable.

The *Pinnularia* cells are **motile.** They commonly progress in the direction of their long axis, either uniformly or by jerks, also turning off now and then laterally from their path. They do not

swim free, but rather creep on some substratum, and it is therefore probable that from the line indicated as a cleft, which we saw in the middle of the valves, a delicate protoplasmic edge is protruded, and forms the organ of movement as a kind of **pseudopodium.**

We now place a preparation of *Pinnularia* on a plate of mica, and heat it over a gas or spirit flame. We then lay the plate of mica when cold upon our object-slide, and observe the preparation dry, but under a cover-glass, with strong magnifying power. We can see that the *Pinnularia* remain as perfect skeletons. With short heating they become somewhat brown, from the carbonized organic substances; with longer continued heating they are colourless. Hydrochloric acid does not touch them; they consist of silicic acid [like flint], and retain and show the finest peculiarities of the cell-wall, which must therefore have been silicified in a high degree. The furrows show in this preparation very clearly as dark striæ, and are besides extremely good for studying the structural relations of the wall. Especially beautifully visible in valve-view are the clefts, which run on both sides from the median nodules to the terminal nodules. Their enlargement at mid-length is manifest. In the girdle-view the edges of the two halves of the cell-wall show clearly; moreover, on the overlapping parts are seen two lines, parallel with one another and with the edges of the valves, which do not extend to the ends of the cell. Flint-skeletons quite as beautiful as these are also obtained if we first allow a drop of concentrated sulphuric acid to act upon our diatoms, and after some time add 20 per cent., and then gradually concentrated chromic acid, and finally remove these reagents with water.[2] Diatom valves which are poor in silex (flint) will neither bear heating red-hot, nor this last method of procedure; they must instead be laid for from four to seven days in hydrochloric acid, to which a little chlorate of potash has been added. In case the valves are still not quite clear and separated, it is advisable after this to lay them for two days in ammonia, and afterwards transfer them to nitric acid.

The remarkable phenomenon of the composition of the cell-wall out of two pieces is, moreover, present in the other Diatomaceæ. Similar motility is likewise observable universally in the free living forms. Even many which grow upon and enclosed in a gelatinous tube, are, if freed, capable of movement, while this appears to be usually wanting in thread-forming species. On

account of the often exceedingly delicate and regular structural relations of their cell-walls, diatoms are much used as **test objects**, in testing the quality of the more powerful microscopic objectives. Especially used are the valves of *Pleurosigma angulatum*, which, with sufficiently strong magnification, shows regularly arranged hexagons.

In order to come to know one of the simplest possible forms of the unicellular green algæ we will examine a *Protococcus*. To this belong in the main all the green incrustations which are found on the stems of trees, damp boards [*e.g.*, wood palings, etc.], walls, and other similar places. In this let us note that it is quite uncertain whether our *Protococcus* is an independent species, or is not rather to be considered a stage in the development of another alga.[3] The form (Fig. 78), which we have removed from an old tree trunk comes under the name *Protoccocus viridis*. We examine this with a strong magnifying power, and find it composed of globular cells, isolated or united into small families (Fig. 78, *A* to *F*). The contents of the cells are bright green, but the whole protoplasm is not uniformly coloured, but rather, as sufficiently strong magnification shows, a number of **chromatophores** are present, which, in lateral contact, occupy the surface of the cell-contents. Where their contact is not complete, the colourless protoplasm comes into view. More or less in the middle of the cell lies the **nucleus**, with its **nucleolus**, which, however, is not usually visible without the help of reagents. The cells have a thin wall, which stains violet with chlorzinc iodine. Numerous cells are usually in course of bipartition by means of a partition wall, which cuts the globular cell in halves (Fig. 78, *D*). The divisions of adjoining cells take place in planes either parallel or cutting one another at right angles. The daughter-cells, becoming rounded off, soon go out of union with one another (*C*, *F*); they

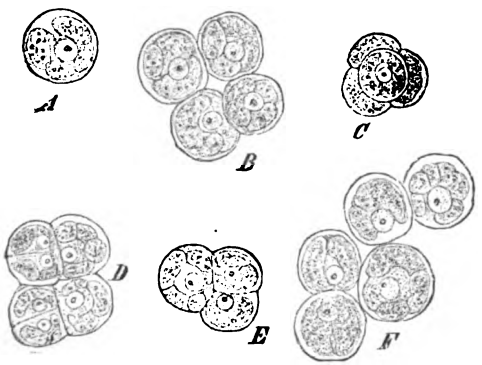


FIG. 78. — *Protococcus viridis*, after treatment with potassium-iodide-iodine. In *D*, the cells on the left have just divided (× 540).

remain, however, for some time clinging to one another, or else become completely separated. If the cells are treated with potassium-iodide-iodine, the nuclei show up clearly (our figures were sketched from iodine preparations). In each nucleus the nucleolus is clearly visible. In the cells which have just arisen by division, the nuclei lie against the young partition wall (*D*). The iodine solution shows small starch-grains in the chromatophores, but no amylum-bodies.[a]

Very simply constructed organisms are met with in the colourless fungal cells hitherto collected together under the name of Saccharomycetes. We provide ourselves with some yeast, the ferment used in brewing beer, and examine a trace of it, diffused in water, under a high power. We find the field of view filled with small cells, individuals of the so-called yeast fungus, *Saccharomyces cerevisiæ*.[4] The cells appear globular or ellipsoid; they have a delicate **membrane**, and in the interior can be recognised a large or several small **vacuoles**, and some strongly refractive granules (Fig. 79, 1). A **nucleus** cannot be distinguished; such is, however, present, and can, though not at all easily, be recognised.[5] For this purpose it is necessary to fix the object with picric acid, in the way given for *Cladophora*, and then to stain with Hæmatin-ammonia. We then find in each cell near the centre a small, round, darker-stained nucleus.—The living object, which we have under observation, shows us numerous cells in course of multiplication. This takes place here in a quite characteristic and peculiar fashion, by the cells forming one, seldom several, small, knob-like swellings, which gradually attain the size and form of the mother-cell, and then can be separated from it (2, 3). In very energetic development we find the daughter-cells united into small occasionally branched chains; in slower development, separation of the cells takes place before any new one begins to form. This is multiplication by **budding**, peculiar to the Saccharomycetes.—In sugar-containing fluids it induces alcoholic fermentation. Recently,[6] the individuality of the Saccharomycetes has been questioned, and they have been declared to be **conidia** (spores of a kind) of different fungi, conidia which have the power, in a suitable nutrient fluid, of multiplying by budding in endless sequence.[b]

FIG. 79. *Saccharomyces cerevisiæ*. 1, not budding; 2 and 3, budding cells (× 540).

We will now turn our attention to one of the Nostocaceæ, of

[a] [b] See notes on page 220.

interest to us on account of its symbiotic * relations with another plant. This last plant, widely cultivated in botanical gardens, is *Azolla caroliniana*. As the *Azolla* winters in plant-houses, we are therefore in a position to obtain material at any time for the investigation of the Nostocaceæ. The Nostocaceæ are, in general, specially disposed towards symbiosis, and we find them in very various plants, especially, however, as constituents of the body of lichens.—The *Anabæna Azollæ*, living in the *Azolla*, is found in definite parts of the plant in question. The leaves of *Azolla* are each two-lobed. The upper lobe is fleshy, and floats on the water; the under is membranous, and immersed. The upper lobe shows in the interior a broad hollow, into which a narrow opening, found on the inner surface of the leaf, leads. This cavity is filled with *Anabæna*, and from the walls of the hollow also grow branched hairs between the coils of this *Anabæna*. In order now to obtain the *Anabæna* for our examination, we pull the upper lobes of some leaves to pieces with the needles, lay on a cover-glass, press upon this a little, and are now pretty sure to find the *Anabæna* strings. This much is certain, that no specimen of *Azolla* is devoid of them. We examine the strings with our highest possible power (Fig. 80). These consist of a row of barrel-shaped cells, which from time to time are interrupted by a larger, ellipsoid or globular cell, the **limiting cell**, or **heterocyst**. The threads are serpentine, coiled here and there, without any visible gelatine coat. The entire content of the vegetative cells is coloured verdigris-green, of the limiting cells is olive green; small, darker-looking granules are distinguishable in these contents; nucleus is wanting. Individual cells are usually found in division (Fig. 80, *a* to *d*). If a twig of *Azolla* is taken between the fingers, and surface-sections taken from it, not infrequently the *Anabæna* can be seen under the microscope in its natural position inside a leaf-cavity. It must, however, have happened by chance, that a leaf-cavity has been cut in the proper

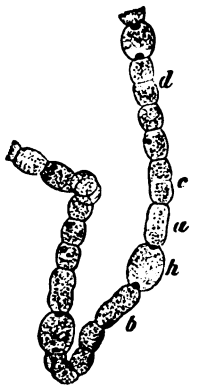


FIG. 80. — *Anabæna Azollæ*, *a* to *d*, successive stages in the division of vegetative cells; *h*, a limiting cell, or heterocyst (× 540).

* Symbiotic, from the substantive *symbiosis*, implying the co-existence in more or less mutual interdependence, of two different organisms. [ED.]

direction. This however frequently occurs; then we see also the segmented hairs which permeate the *Anabœna*.

Quite similar is the structure of the threads in the olive-green lobed gelatinous masses, sometimes found in great masses on paths, and which belong to *Nostoc ciniflonum*, Tournefort (*commune*, Vauch).[7]

In examining any terrestrial form of *Vaucheria*, particularly that collected from flower-pots, we meet with *Oscillaria*, likewise belonging to the Schizophyta (splitting plants), in closest affinity to the Nostocaceæ. They are found, moreover, almost everywhere in standing water, on muddy ground, and under similar conditions. Their presence is often betrayed by an unpleasant muddy smell. Cultivated in vessels, they creep in part to the walls of this, over the surface of the water. They are nearly straight, or even coiled threads, coloured from blue-green, verdigris-green, olive-green to brown; can, however, be colourless, and in many forms distinguished by active motility. The threads are free, or enclosed in a gelatinous sheath. They can be inserted individually, or in numbers, in such a sheath. The sheaths arise from the outer layers of the membrane of the threads; where these layers become fluid, the sheaths are wanting. The threads are divided by cross partition walls into a multitude of similar short cells. The partition walls in some species can be seen very easily, in others with great difficulty. With the exception of this difference, there is great uniformity in the structure of these organisms. The cell-contents are, in general, coloured throughout the entire mass; no nucleus can be recognised in the interior, but numerous small granules. The granules are either distributed through the entire cell-contents, or are specially collected at the partition walls. It matters not what species is chosen for examination, but preference should

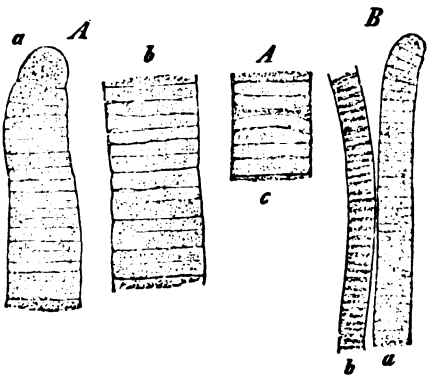


FIG. 81.—*A, Oscillaria princeps; B, Oscillaria Froelichii; a*, ends of the threads; *b*, piece from the middle of the thread; in *B*, *b*, the granules collected against the partition walls; in *A*, *c* is a dead cell between two living ones.

be given to the thicker forms, with clearer partition walls, as represented in Fig. 81.[c]

The phenomena of movement, as we must have noticed from the very beginning of our observation of the Oscillariæ, are very interesting. Especially in the thicker forms, with somewhat bent end and distinct granules, and with a sufficiently strong power, we shall be able accurately to study the phenomenon. We then determine that with the movement of the thread is combined a slow rotation on its axis. Simultaneously the thread shows irregular flexions, or **nutations**, which are the expression of existing differences in the intensity of growth on its different sides. These flexions usually take place slowly; can, however, induce violent movements when the flexion is stopped by some obstacle, and then by overcoming this, the tension is suddenly equalized. The Oscillaria-threads move now forwards, now backwards. The movements can only take place when the threads have a point of support on some other object. The straight threads move like those which are bent; in these latter the phenomenon is, however, especially striking, and at once visible, while in the straight threads it is necessary to fix the attention upon the individual granules of the surface, in order to demonstrate a rotation of the thread on its axis. The origin of the movement is not yet known with certainty; it has recently been maintained that it is occasioned by protoplasmic processes [whether pseudopodia or cilia], which pass through the membrane to the exterior.[8]

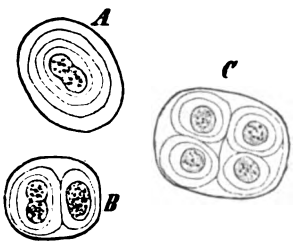


FIG. 82. — *Gleocapsa polydermatica.* In *A*, at the commencement of division; in *B*, to the left, shortly after division (× 540).

To the same class of organisms as the Nostocaceæ and the Oscillariæ, belong the still simpler-constructed Chroöcoccaceæ, which we will study upon one of the widely-distributed species of *Gleocapsa*. We choose *G. polydermatica** (Fig. 82), growing upon damp walls or rocks, recognisable from their dirty green to olive colour, and their firm, clearly and repeatedly layered, gelatinous envelopes. Other species, with less beautifully laminate

* More readily obtainable, and very like, is *G. caldariorum*, a species growing commonly on the walls, flower-pots, and glass, etc., in conservatories and greenhouses. [ED.]

[c] See note on page 220.

gelatinous envelope, will serve the same end. In all of them we find in the gelatinous envelope uniformly coloured cells, more or less clearly granular, and devoid of nucleus. By these peculiarities of their cell-body the Chroöcoccaceæ are distinguished from Protococcaceæ and especially Palmellaceæ, which in many forms very strongly resemble them, but which have a nucleus, and chromatophores, separated from the rest of the protoplasmic body.

In *Gleocapsa polydermatica* the cell-bodies arisen from just previous division are quite globular (Fig. 82, *C*). They then begin to grow in length, and become ellipsoidal. They then show a weak hour-glass-like constriction (*A*) in mid-length, after which a delicate partition wall becomes visible at this place. The daughter-cells now round off towards one another, and, by swelling of the separating wall, and the thickening layers afterwards developed, become thrust back from one another. Owing to the ever-new development of gelatinous layers in the interior, the older ones become stretched, finally ruptured and cast off[9] [often parts of such layers are found, while the rest have disappeared]. A considerable number of generations is therefore combined by the gelatinous envelopes into a single cell-family or **colony**, whence often called **colonial algæ**. By rupture of the outer envelopes the families fall apart. An isolated cell is rarely found, and then is usually surrounded by a considerable number of gelatinous envelopes (Fig. 82, *A*). In such cases the cell-division is discontinued, not the thickening of the wall.[d]

We have therefore found that in Nostocaceæ, Oscillariæ, and Chroöcoccaceæ, the cell-contents differ from those of the plants hitherto considered by us: while in these latter we find the separation of the protoplasm into cell-plasma, nucleus, and chromatophores, we find here all these elements of the cell-body still united into a single substance.[10] Distinguished by their coloration from the pure green of other plants, these plants have been collected together under the name of Phycochromaceæ, or Cyanophyceæ. The simplicity of organization of these organisms is betrayed also by the absence of **sexual multiplication**. One kind of **asexual multiplication** is, however (often by the side of other kinds of asexual multiplication), quite peculiar to them, viz., that by vegetative bipartition; and therefore these organisms have been called segmenting or splitting algæ, or Schizophyceæ.[11] Recent researches[12] suggest that the thread-like Schizophyceæ are capable of separating into globular cells surrounded by

[d] See note on page 220*a*.

gelatinous layers; *i.e.*, of entering into a chroöcoccaceous condition, like to *Gleocapsa*. An analogous phenomenon, found amongst the green Algæ in the case of Protococcaceæ, gave rise to the question whether *Protococcus viridis* was an independent organism. This question, therefore, repeats itself with the Chroöcoccaceæ, which are perhaps merely developmental stages of the thread-like Schizophyta.

NOTES TO CHAPTER XX.

[1] Compare Pfitzner, in Hanstein's *Bot. Abhand.* Bd. I., Heft. II., p. 40, and Schenk's *Handbuch der Botanik*, Bd. II., p. 410. In the former the literature is given.

[2] Miliarakis, *Die Verkieselung*, Würzburg, 1884.

[3] Compare especially Cienkowski, *Botan. Zeitung*, 1876, Col. 17, and *Mélang. biol. de St. Pétersbourg.*, tom. IX., p. 531.

[4] Reess, *Alcoholgährungspilze*, 1870.

[5] Schmitz, *Stzber. d. niederrh. Gesell.*, 4th Aug., 1879.

[6] Brefeld, *Botan. Unters. über Hefepilze; der Schimmelpilze*, V. Heft, 1883, p. 178.

[7] Compare Thuret et Bornet, *Notes algologiques*, II., p. 102.

[8] Engelmann, *Botan. Zeitung.*, 1879, Col. 49.

[9] Schmitz, *Stzber. d. niederr. Gesell.*, 6th Dec., 1880.

[10] Schmitz, *Die Chromatophoren der Algen*, p. 9.

[11] Compare, for example, Falkenberg in Schenk's *Handbuch der Botanik*, Bd. II., p. 304.

[12] Zopf, *Bot. Centralbl.*, Bd. X., p. 32; *Zur Morphologie der Spaltpflanzen*, 1882.

[*Notes to page* 215.]

[a] We have said that this alga is by some considered to be only a stage in the life history of some higher form, probably one of the Volvocineæ. If some of this material is placed in water and exposed to the light, some of the cells will enter upon another phase of their existence, becoming motile by means of cilia. More suitable for microscopical study, however, is another form of *Protococcus*, commonly known as *Protococcus* (or *Hæmatococcus*) *pluvialis*. This *Protococcus* is found very widely distributed in rain-water, in the mud at the bottom of open water-butts, in roof-gutters, etc.

[b] Yeast can be grown for laboratory purposes, either in the bulk or in moist-chamber cultures, in "Pasteur's fluid." In slide cultures very beautifully branched chains of cells can be obtained. The following is the simplest way of preparing Pasteur's fluid:—Keep dry in a bottle, ready mixed and finely pulverized, 20 gram. Potassium phosphate, 2 of Calcium phosphate, 2 of Magnesium sulphate, and 100 of Ammonium tartrate. For use, dissolve in the proportion of 1 gram. of this mixture with 12 of sugar, in 70 cc. of water. [Ed.]

[*Note to page* 218.]

[c] By the use of 1 per cent. chromic acid, or of concentrated picric acid, the threads are fixed, and with the subsequent use of logwood they can be stained, and the grains show up very clearly.

[*Note to page* 219.]

[4] With careful examination we can in general determine that the number of the thin cell-walls which their stronger refractiveness has made visible, does not commonly correspond with the number of cell-generations enclosed within. Usually, it is true, the formation of one such cell-wall f llows the division of the cell-contents; nevertheless, two or more such cell-walls can be intercalated in the gelatinous wall between two stages of division.

CHAPTER XXI.

SCHIZOMYCETES* (BACTERIA). USE OF IMMERSION OBJECTIVES.

MATERIAL WANTED.

Some green leaves, *e.g.* those of the lettuce. Fresh.
A carrot, turnip, or potato.
Some peas, dry or green.
Vaccine lymph, best in capillary glass tube.
Hay.
Beggiatoa alba, p. 232, can usually be obtained freely upon pieces of indiarubber tubing kept in water. [Ed.]
(Other materials may be available in addition to these.)

LET us now turn our attention to some examples from the group of the smallest known organisms, the **Bacteria,**[1] in order to obtain some information as to the general form which they assume. We shall not endeavour, in the first place, to study any particular species; we will rather leave it to the accident of what form happens to be at our disposal. We boil some green leaves, say lettuce leaves, in a Florence flask, and leave it standing open at a comparatively high temperature. Into another flask we place some peas—killed by steeping in boiling water—with a little water. At the same time we distribute disks of boiled carrot, turnip, and potato, on watch-glasses or object-slides, and place them about in warm, moderately moist places; some free, others covered with glass bell-jars. Upon the decoction or infusion of leaves after two days a skin may have been formed, which we will call the **pellicle.** On the different vegetable disks we see small whitish, rarely coloured, masses of gelatinous substance appear. If we bring a trace of such a mass of jelly into a drop of water on an object-slide, and examine it with the strongest possible magnification, we find an enormous number of exceedingly minute bodies, appearing almost dot-like, imbedded in the jelly. These bodies show a necklace-like arrangement; we find them singly, or in pairs, or united in large number into a thread. Embedded in the

* Segmenting, splitting, or fissing fungi. As a convenient term, not implying any individual kind, I shall use the word *bacteriad*. [ED.]

jelly, therefore, we have the **Coccus**-form of a bacteriad. If we wish to define the outer limits of the jelly, which, in its refractive relations, differs only little from water, we can readily accomplish it with the aid of Indian ink.[2] The ink must be of good quality, and should be ground down very carefully in water. A drop of this ink can then be placed upon the object-slide, the gelatinous mass which is to be investigated placed upon a cover glass, and the cover-glass then laid upon the drop. In this way the particles of ink are prevented from passing between the jelly and the cover-glass. The limits of the jelly are now sharply defined, on account of the surrounding fluid being filled with fine particles of ink, which exert no injurious influence on the object. Such masses of bacteria embedded in jelly are distinguished as **zoogloea** [or the **zoogloea stage** of the bacteriad]. The jelly arises from the swollen membranes of the bacteria. In the bacteria of putrefaction these membranes are composed of a peculiar albuminous substance, **mycoprotein**; with bacteria not provoking putrefaction they consist of **cellulose**. We make use of the property of bacteria of eagerly taking up certain aniline and azotic colours, in order to stain them. For this purpose we only need to add a little methyl violet, gentiana violet, methyl blue, fuchsin, Bismark brown, or Vesuvin, to the preparation. Hæmatoxylin (logwood) at the same time colours the jelly; and we therefore use this in order to make the jelly distinct. We will confine ourselves at first to gentiana violet, which stains bacteria with extraordinary rapidity and intensity. We then see the bacteria very clearly, and can form an opinion as to their mode of multiplication, which takes place by successive bipartition or fission. This multiplication, in contradistinction to the "budding" of the yeast fungi, has given to the bacteria the name of "splitting or fissing fungi," or **Schizomycetes**.—It is quite possible that the jelly taken under our observation does not contain round "Cocci," but **rodlets** (compare Fig. 85 *A*, on p. 237). The rodlets can be identified as composed of shorter segments, which stand out very clearly if we add iodine-solution to the preparation. The segments now appear much shorter than they appeared in the fresh state; partition walls are now shown which formerly were invisible.[a]

Some bacteria are distinguished by the fact that in the stages preceding spore-formation they form a starch-like substance in their body, and then, on the addition of iodine solution, colour blue or violet.

[a] See note on page 215a.

In the pellicle which has formed upon the leaf-infusion (cf. Fig. 85, *A*, page 237) we have also a form of zoogloea, in which the cell-rows are held together by a jelly into a superficially developed skin. This is seen to be traversed by fine, undulating partly parallel threads, formed of *cocci* or, more commonly, of rodlets. The segmentation of the rodlets is again made specially clear by the addition of iodine-solution. In such material obtained by cultivation, the **swarming** stages of development will often be found. We are almost certain to find such in the water in which the peas have been soaking for one or two days. We then see the bacteria in dancing movement, now forwards now backwards, hurrying about in different directions. Exceedingly fine *cilia* have been repeatedly demonstrated as the cause of this movement (Fig. 85, *B*); in other cases these have not been found.

If we examine the pellicle of a leaf-infusion which has already stood for some time, we shall find the rods or threads ultimately in course of formation of **spores** (Fig. 85, *C*). The contents of the rods have aggregated at one or several points, and produced rounded or elliptic highly-refractive structures, which appear as darker bodies, and are *resting-spores*. These persist, while the emptied membrane of the rodlets is finally disintegrated. In material from other cultures we shall quite as commonly find rodlets which have formed but one resting-spore, at one end, and have thus taken the form of a pin or tadpole. Such forms, for example, are very frequent in the very widely-spread bacteria of butyric fermentation (*Clostridium butyricum*).[a]

As the Bacteria are the smallest of known organisms, for a more complete study of them the most powerful and best objectives, and the most favourable possible illumination, are alike necessary. As objectives, those for *homogeneous immersion* are especially to be recommended; while the most advantageous conditions of illumination can be attained by the aid of an achromatic condensor, or apparatus such as Abbe's "Beleuchtungs-Apparat." In most cases, however, *water-immersion* objectives will suffice. Objectives for water-immersion, as well as those for homogeneous immersion, can be used with any of the microscopes we have heretofore specified; but a condensor, or other similar apparatus, can only be added to a microscope specially arranged for its use. They require, as was indicated in the Introduction, one of the larger microscope stands.

If a water-immersion lens is employed, cover-glasses of a definite thickness, indicated by the optician (compare Introduction), must

[a] See note on page 215*a*.

be used. If the objective is provided with a "correcting screw," the objective is regulated for any thickness of the cover-glass, so far as this lies within the possible range, by turning the correcting screw placed in the upper part of the objective. Every objective provided by an optician has usually a definite value to its screw for each hundredth of a millimetre thickness of the cover-glass. In order to use the objective, a small drop of distilled water is placed on its front lens. Care must be taken that this drop of water is not allowed to dry; between the cover-glass and objective, however, it is so protected against evaporation that it will usually last for several hours. In moving the object-slide, care must also be taken that the drop of immersion-fluid does not go to the edge of the cover-glass, and so mix with the fluid in which the object is immersed. If this should happen, the objective should be at once cleaned, and the fluid on the cover-glass removed. In case an object, already covered with a cover-glass, is examined with the water-immersion objective, and the thickness of the cover-glass is not known, the correction, if necessary, must be arranged during the observation. While observing, we turn the ring towards the one and the other side, and compare the effects produced. As the correcting screw in almost all objectives is so arranged that the front lens remains immovable, and only the upper lenses of the objective are moved, the object remains, during the correction, approximately focussed. The correction is complete when the figure appears sharpest.

Objectives for "homogeneous" immersion have no correcting screw, and the thickness of the cover-glass, within the permissible limits, matters nothing. For these a drop of the immersion fluid provided by the optician (oil of cedar-wood, or a mixture of oil of fennel and castor oil, or zinc-iodide glycerine) is placed upon the front lens of the objective. We take the smallest possible quantity of the immersion fluid; as this does not evaporate, it need not be replenished during the observation. As with the water-immersion, we must take care that in moving the object-slide the immersion fluid does not run to the edge of the cover-glass. For cleansing the objective after use, a very clean and often-washed piece of linen is best. In order to clean the cover-glass, we use a piece of linen moistened with chloroform. As objectives for homogeneous immersion bear a change of eye-pieces very well, we can procure a complete series of these.

In case the observer has a larger microscope stand, to which as

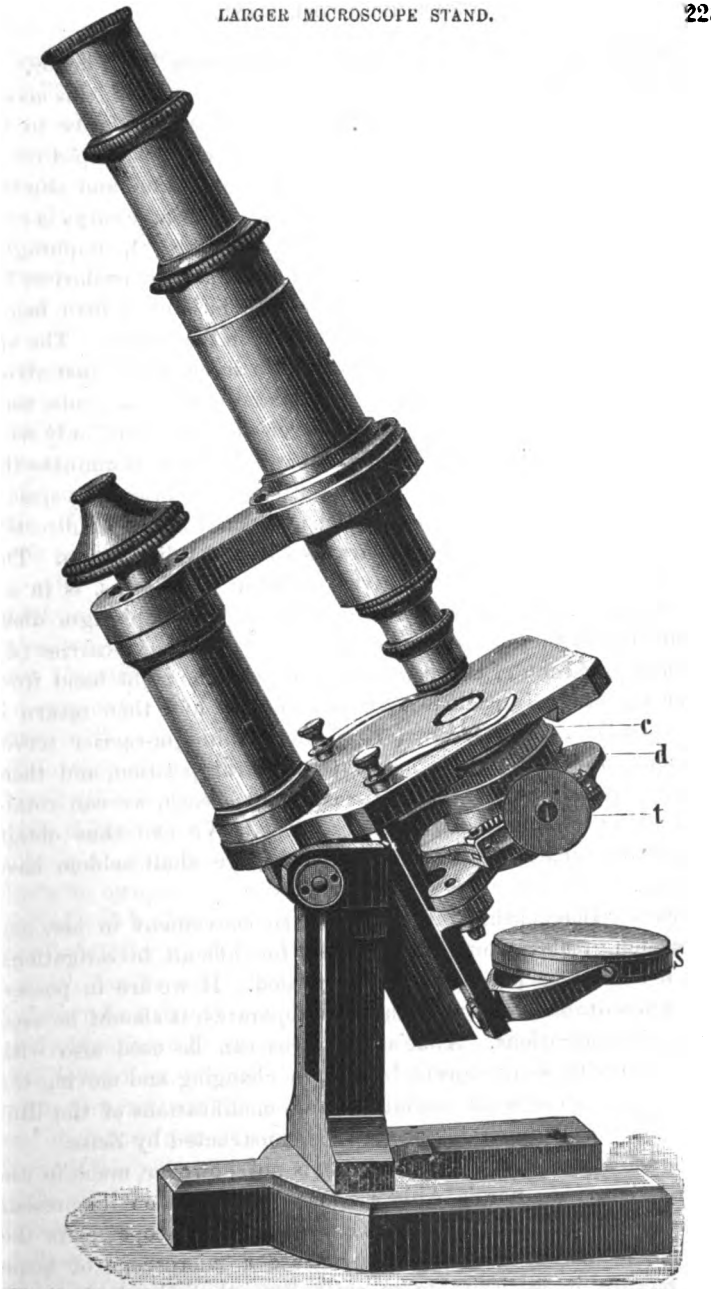


Fig. 83.—Stand Va of Zeiss, $\frac{2}{3}$ actual size, with Abbe's "Beleuchtungs-apparat." *c*, Condensor; *d*, Diaphragm-bearer; *t*, Screw to this; *s*, Double mirror.

e.g. in that illustrated in Fig. 83*, a "condensor" can be, or, as in Fig. 83, an Abbe's "Beleuchtungs-Apparat," is fitted, this accessory apparatus can now be brought into use. In order to fix Abbe's apparatus, the body of the microscope must be sloped (even more than in Fig. 83), remove the ordinary mirror, and slip the apparatus in its place in the same groove. The apparatus is constructed in one piece, and consists of condensor (*c*), diaphragm-bearer (*d*), and double mirror (*s*). The apparatus is pushed so far up that the upper surface of the condensor lies only a little below the upper surface of the stage (as is shown in the figure). The apparatus is then fixed in the groove with a screw found just above the mirror. Of the two mirrors the plane one is, as a rule, used with the apparatus. The concave mirror should be used only with very weak objectives, if the plane mirror does not illuminate the whole field of view quite equally. With the exception of a special case of bacteria investigation, which we shall speak of directly, we ought not to use Abbe's apparatus without a diaphragm. The narrowest diaphragm which gives sufficient brightness, is in all cases the best. In order to bring into use the diaphragm disks provided with the instrument, we turn the diaphragm-carrier (*d*), which is found under the condensor, away to the right hand from under the stage, introduce a diaphragm-disk, and then return it into its place. The screw (*t*) on the diaphragm-carrier serves to remove the diaphragms out of the central position, and then, as the diaphragm-carrier also turns in its sheath, we can rotate them about the axis of the microscope. We can thus obtain oblique illumination; to which, however, we shall seldom have recourse.

Abbe's "Beleuchtungs-Apparat" is so convenient in use, and offers such great advantages, especially for difficult investigations, that it cannot be too highly recommended. If we are in possession of a suitable stand with such an apparatus, it should be used for all investigations. Abbe's apparatus can be used also with advantage with weak objectives, and, by changing and moving the diaphragms, permits all gradations and modifications of the illumination. The apparatus as above is constructed by Zeiss.

This microscope of Zeiss (Fig. 83), is not, however, much in use outside Germany; nor is Abbe's apparatus suited, on its present lines of construction, for use with English microscopes.—As the English student will probably purchase a microscope of home manufacture it is desirable to state here that the larger, and

typically English, stands are not to be recommended for student use. Their length of body makes it exceedingly difficult to use them upright without a special table; and the upright position is, all round, the more convenient for student work. Nor are mechanical appliances for moving the object-slide about on the stage of utility commensurate with their cost, and the want of independence which they induce. Most of the English makers manufacture microscopes with tubes of about the Continental length, but of better workmanship than the ordinary Student stands, and suited for the addition of accessory illuminating and other appliances. Without desiring to assert or imply its absolute superiority, where at least three or four excellent instruments exist, the "Student's Stand" of Ross (monocular) may be looked upon as typical of instruments of this class, in which workmanship of the highest excellence is combined with simplicity and moderate price. It is illustrated in the accompanying Fig. 83*. The model shows peculiar lightness and stability. The body is swung between two pillars, so that it can be removed from the vertical to any extent in the backward direction, and can even be thrown a little beyond the vertical in the forward direction. It has coarse rack-work adjustment of considerable delicacy, and a fine adjustment. The eyepieces are of Continental size. The stage rotates upon a centre in the optic axis of the instrument. The mirror slides up and down in a groove upon a "swinging tail-piece" (Zentmayer's) which replaces the jointed arm of most mirrors, and enables rays of any degree of obliquity to be thrown upon the object; and the mirror can even be swung round above the stage so as to illuminate an opaque object from above. The same tail-piece carries a cylindrical bearer (seen in the figure between mirror and stage), likewise sliding up and down in the same groove with the mirror, in which can be placed a condensor or other accessory apparatus. If the student should not wish to purchase a special condensor, a small adaptor can be obtained, by means of which his one-inch, or other low-power, objective can be screwed into the carrier, front lens uppermost, and then, after sliding in the groove till its proper focal distance from the object, as ascertained by experiment, is attained, this objective will serve as a thoroughly good condensor. As the condensor is never required excepting with high powers, the low-power objective will always be at liberty for this purpose. In place of the diaphragm wheel, as usually supplied, an "Iris-diaphragm," which, by simple movement of a lever arm, gives a

FIG. 83*.—Student's monocular stand of Ross, with concentric rotating glass stage, one eye-piece, etc., as in above figure, but no objective. Price, £5 18s. 6d. About ⅓ actual size.

nearly circular aperture of any chosen size, can be added at an extra cost of about £1.

In order to be able to work with the microscope in dull weather, or in the evening in general, it is an advantage to have a lamp with a wide burner, and to shade between this and the mirror of the microscope by means of a plain glass water-bottle, or the largest size possible of Florence-flask, filled with a very dilute solution of ammonio-cupric oxide. Microscoping in the evening exhausts the eyes very little, provided care be taken that the surroundings are illuminated just as brightly as the field of view of the microscope.

Small incandescent electric lamps have been recently recommended as sources of light; a current from about three Bunsen elements, each about eight inches high, suffices. It is best and simplest to place the incandescent lamp in front of the microscope, and between this and the mirror of the instrument to place a globe (as above) filled with very dilute ammonio-cupric oxide. The comparative richness of the incandescent light in rays of short wave length, although far less marked than that of the arc light, makes it very suitable for the study of delicate structural relations.

As already noted, methyl-violet, gentiana-violet, methyl-blue, fuchsin, Bismarck-brown, and Vesuvin, are especially serviceable for staining bacteriads. These stains are best used in watery solution, which must be either fresh, or at least freshly filtered. For this purpose we keep a saturated alcoholic solution of these colours ready, and add one of them drop by drop to a large quantity of distilled water. Bismark-brown and Vesuvin, however, as they are altered in alcohol, must be kept in watery solution, and this must be filtered before each use. The bacteria found in a fluid medium should be spread in the thinnest possible layer on the cover-glass, and allowed to dry at the temperature of the room. If the fluid contains albuminous bodies, or mucilage, these must, after the preparation is completely dried, be fixed either by laying the cover-glass for several days in absolute alcohol, or, still simpler, by a higher temperature. For this purpose we pass the cover-glass pretty quickly several times through the gas or spirit flame, during which the surface covered by the bacteria should be turned upwards. We stain it by spreading over the cover-glass, prepared in this or any way, but which in all cases must be dry, a drop of colouring fluid, and allow it to act for from five to ten minutes. Or we stain it in a saucer, which contains a larger

quantity of the colouring fluid, on which the cover-glass is allowed to float for from ten to thirty minutes. Warming the fluid from 30° to 60° C. (86° to 140° F.), hastens the operation. After complete staining, the cover-glass is washed in distilled water, dried at the temperature of the room, a drop of oil of turpentine, xylol, or cedar oil placed upon it, and the examination carried on in this. If the preparation is to be preserved, remove the oil with blotting paper and imbed in dammar or in Canada balsam, which, however, must be dissolved in turpentine and not in chloroform. Care should be taken, in case the preparation should be later on examined with a homogeneous immersion objective, that the dammar or Canada balsam does not come out from the edge of the cover-glass, since both of these are soluble in the immersion oil, and the whole cover-glass would thus be soiled. This inconvenience can also be obviated by making at the edge of the cover-glass, after the dammar or Canada balsam has become dry, a border of black varnish, or of gold-size. To do this, a small camel-hair brush is used, and care is taken that the varnish does not run over the cover-glass more than is necessary.

If the preparation happens to be overstained, a result brought about with especial ease when the colouring fluid is warmed, a portion of the colour can be removed by allowing absolute alcohol (quite free from acid) to work upon it for a sufficient length of time. Or the same result can be attained by using oil of cloves for the purpose of "clearing" the preparation, for this oil extracts more or less of the colour, according to the length of action. Overstained and then partially decolorised preparations are often the most beautiful. Chloroform would remove the colour, and hence must not be used as the solvent for Canada balsam in permanent mounting. For the same reason the Canada balsam must not be used warm. For permanent mounting in Canada balsam some degree of overstaining is an advantage, as the balsam slowly extracts some of the colour.

In the examination of fluids for bacteria, it must, however, be noted that different kinds of granulation may be present, which make observation more difficult, and may even be deceptive. To remove these we make use of the resistance of bacteria to dilute mineral acids, to acetic acid, and to weak alkalies. We use 50 per cent. acetic acid, or 12 per cent. sulphuric acid, or, what is still better, 3 per cent. potash solution. In this last the preparation becomes at once as transparent as is needful, the bacteria

stand out prominently, and increase in size somewhat through swelling, so that we can even make use of less strong magnifying powers. Since the larger fatty masses, in case they are present, interfere with observation very seriously, it is necessary to take care to remove them. This can be effected either by covering the dry preparation with a drop of potash, and warming it over a flame till bubbles begin to form, whereby the oil is converted into soap, or the dry preparation is manipulated for a few minutes in a watch-glass of chloroform, and afterwards with absolute alcohol, and after this latter has evaporated, potash applied.

A concentrated solution of pure dry iodide of zinc in pure glycerine can also be used as a fluid for immersion objectives. This, after filtering, if necessary, is evaporated in a water-bath to the refractive index 1·518 (for line D of spectrum). This fluid does not attack balsam in settings of the cover-glasses, and has the further advantage that it can easily be washed off from the cover-glass with water.[4] Preparations stained with Bismarck-brown or with Vesuvin retain their colour also in glycerine, and can therefore be preserved in it. The closure of the edges of the cover-glass can be effected with Canada balsam in chloroform. After some days or weeks, just as is convenient, the Canada balsam can be covered with a layer of gold-size or of varnish, not in order to prevent the immersion-oil from attacking the closing cement but because it is recommended especially as very durable. Canada balsam in chloroform is apt, when dry, to spring. Hence the advantage of turpentine, since the fat is left behind in drying, and the balsam never becomes brittle. There is a concurrent disadvantage, however, that the slides must not be pressed one on another for any length of time, or they may stick together. Nor must the proportion of fat in the balsam be allowed, by adding repeatedly more turpentine to dilute it in its bottle, to become considerable. Covering with gold-size is advisable.

If one of the larger forms of bacteria is under examination, we may also, with the aid of our strongest objective and the most successful staining, learn something about the contents of the cells. These appear to be a homogeneous plasma, in which can be embedded finer or coarser granules, which probably consist of fat. Nuclei cannot be identified even in the largest forms. Only in rare cases are the bodies of the bacteria coloured while in living condition.

If we have at command water in which algæ, especially *Spirogyra*

and *Vaucheria*, are decaying, and examine a little of this fluid, we shall find in it, almost to a certainty, motile, exceedingly fine spiral threads (Fig. 84). These flexible, corkscrew-like threads move rapidly in the water. They turn on their axis, and at the same time bend to and fro. Individuals suddenly stand still, then hasten on again. The spirals found under such circumstances in all probability belong to *Spirochæte plicatilis*, the *Spirochæte* of marshes. If these spirals are allowed to dry, and stained, we shall see that they are not unicellular, but consist of successive segments, which may vary in length according to circumstances. Very fine Spirochæte can usually be obtained by keeping in a warm place a filtered infusion of pea-flour.

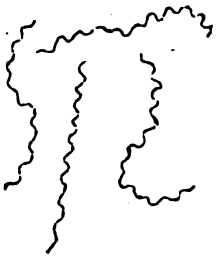


FIG. 84.—*Spirochæte plicatilis*, after aniline staining, partially showing the segmentation into rootlets (×510).

On the same decomposing algæ, or on pieces of other decomposing aquatic plants, or other similar substratum, we commonly see growing fine threads, which are *Beggiatoa alba* (Vauch).[6] These bacteriads are especially widely diffused in water which receives the refuse from factories, and in sulphur springs. They then often cover the masses of mud on the bottom and sides with a dirty-white layer. They are amongst the largest known bacteria, and can be distinguished with even comparatively low magnification. The threads are of variable thickness (from 0·001 to 0·005 mm.), are attached or free, the free however only being parts of those attached. A segmentation of the threads into shorter or longer rodlets is more or less distinct; the cell-contents are usually distinguished by a greater or less number of strongly refractive grains. If we allow the preparation to dry, and run in bisulphide of carbon, the grains are dissolved; they consist of sulphur. In threads very rich in sulphur the segmentation is indistinct, and only appears after aniline staining, or after heating in glycerine or in sulphite of soda. By the glycerine the grains are partly, by the sulphite of soda completely, dissolved. By cross segmentation the threads can separate into *cocci*, and it has been observed that, in thicker threads, even a quadripartition of the cells can result from this cross division. Moreover, "swarming" *cocci*, rodlets, and spirals have been observed as developmental stages of *Beggiatoa*. The attached threads can, in their upper parts, be spirally bent. The straight as well as the spiral frag-

ments of threads are flexible, and show creeping movements. These characters serve to connect *Beggiatoa* with the *Oscillatorians*. *Beggiatoa* separates out the sulphur compound from the water in which it dwells, and thus sets free a more or less considerable quantity of sulphuretted hydrogen. *Beggiatoa alba* can be readily grown on pieces of grey india-rubber tubing kept in water. It will usually have formed a good coat in about a couple of months.

We will now turn our attention to another object, which shows the *coccus*, rodlet, and spiral, and at the same time the thread form, combined. For this purpose we will use the white "fur" of teeth. If a little is diffused in a drop of water, and examined with the strongest possible magnification, we shall see long, ap-

FIG. 84*. Bacteria of the fur of teeth. *a*, *Leptothrix buccalis*, in *a** after action of iodine; *b*, Micrococcus; *c*, Spirillum dentium, after action of iodine; *d*, "comma bacillus" of the mucous membrane of the mouth (×800).

parently unsegmented threads, of various lengths and thickness, grouped together into close bundles (Fig. 84*, *a*). These threads and rodlets have been described as *Leptothrix buccalis*, Robin, but it has not yet been determined with certainty that they belong to a single definite species. If the preparation be treated with iodine, the threads appear as composed of short segments; not infrequently the contents, especially of the thicker threads, assume a blue colour. These threads are always embedded in dense, gelatinous, irregular balls of micrococci (*b*). Between them we can usually see spiral Spirilla in active movement. These are *Spirillum dentium* (*c*). More rarely, amongst all these forms are also found thin, arched, relatively short rodlets (*d*), mostly in active jumping movement, which have been identified as the

"comma" bacilli of the mucus of the mouth. All these bacteria live as saprophytes on the mucous membrane or the fur of teeth, but are also concerned in the decay of teeth, in that they penetrate into the calcified tissues of the teeth, and destroy the softened bone.

In *Beggiatoa alba* we have an example of a **pleomorphic species** of bacteriad, into the developmental circle of which various forms enter,[9] which we have recognised as Micrococcus, Bacterium, Bacillus, Vibrio, Spirochæte,[8] etc. But it appears certain that pleomorphism is absent from the greater number of bacteria, and that these assume the same form under the most diverse cultural conditions. The globules or ellipsoidal forms we call *cocci*; rods, threads, and screws have a corresponding form. The shorter rodlets are distinguished as *Bacteria* from the longer *Bacillus*; the simple threads as *Leptothrix*, the branched *Cladothrix*; the spirals with comparatively wide turns and greater thickness of the threads are called *Spirillum*; or, if they contain sulphur, *Ophidomonas*; spirals with elongated turns, *Vibrio*; very thin spirals, with small diameter and also smaller distance of the turns, *Spirochæte*; riband-like tapering spirals, *Spiromonas*; flexible spirals, whose ends coil back towards one another, *Spirulina*.[10]

We have seen in the study of the segmenting algæ that they also are distinguished by like variability of form in the different stages of development; and the comparison of the bacteriads with these segmenting algæ leads, in fact, to the presumption of some close relationship between these organisms. In the segmenting algæ also we have made the acquaintance of *cocci*, rodlets, threads, and spiral forms. Moreover, we have met with the phenomena of movement amongst them, and even in their ability to resist high temperatures the segmenting algæ approach the segmenting fungi. The first plants which show themselves in hot springs are segmenting algæ, though it is true they do not resist so high temperatures as, *e.g.*, the spores of the bacteria of hay, whose capabilities for germination temporary boiling seems only to heighten. Moreover, in the structure of their cell-body, segmenting algæ resemble segmenting fungi, since both groups are devoid of nucleus and of separated chromatophores. To this we may add the mode of vegetative multiplication, which gives to the two sections their respective names. For these reasons we can consider the segmenting fungi to be a colourless section of the segmenting algæ, or, at any rate, one devoid of a colour which enables carbon-assimilation,

and which, together with the segmenting algæ, form the class of segmenting plants, the **Schizophyta.**

Bacillus tuberculosis, of recent times considered to be the cause of tuberculosis in the sputum of consumptives,[11] is always motionless, very minute, somewhat tapering at its ends, and now and then with four to six grains, which are considered to be spores, in the interior. This *Bacillus* is distinguished by special relations towards staining reagents, which render it possible to distinguish it from other *Bacilli*. The substance to be tested is spread as flat as possible upon a cover-glass, and allowed to dry at the temperature of the room. The albumen present is then fixed by passing the slide bearing the cover-glass three or four times through a spirit or gas flame, the preparation side turned upwards. We then saturate a quantity of water with aniline by shaking up the water with an overplus of this body. We filter it through paper previously damped with distilled water, and add to 100 parts (by measure) of the fluid, drop by drop, 11 parts of a saturated alcoholic solution of fuchsine or of methyl violet, and then 10 parts of absolute alcohol. This staining fluid can be preserved for at least ten days in a well-closed glass, without its being necessary to filter each time it is used. The cover-glass is now allowed to float for half a day in this fluid. The staining proceeds more quickly if the solution is warmed till evolution of bubbles commences. The action need then only last ten minutes. After this the cover-glass is laid for, at the most, a half-minute in a solution of 1 part nitric acid to 3 or 4 parts distilled water, and then for some minutes in 60 per cent. alcohol. The entire preparation is thus coloured, with the exception of the tubercle bacilli, if any such are present. The preparation is then observed in water; or it can be washed in water, allowed to dry, and afterwards mounted in Canada-balsam dissolved in turpentine.—The material for preparation of sections must be well hardened in absolute alcohol, or, if hardened in other ways, must lie a long time in alcohol. The sections are then stained in the same way as above described. They must remain in the staining fluid at least twelve hours. After being passed through 60 per cent. alcohol, they can be placed for some minutes in dilute watery solution of Vesuvin or methyl-blue. They are then once more washed in 60 per cent. alcohol, passed from thence into absolute alcohol, in order that, when completely deprived of water, they may be placed in oil of cedar (which does not extract the aniline

colours from the preparation), in which they can be examined. In order to preserve them, the preparations are mounted in Canada-balsam dissolved in turpentine.[12] Tubercle bacilli are beautifully visible with magnification of 300 diameters.—These bacilli are likewise stained with great intensity by fuchsin prepared in the following manner:—In 100 gram of a 5 per cent. watery solution of carbolic acid is dissolved 1 gram fuchsin, and then 10 grams alcohol added. Filter. The solution keeps well. It is advisable to warm the fluid in using it.[12*]—For bacteria found in fluids double-staining has also been employed. According to one of these methods,[13] the fluid diffused on the cover-glass is dried, and fixed with osmic acid vapour, or with a 0·5 per cent. solution of chromic acid. It is then washed with distilled water, and stained, usually for from half an hour to an hour, with 0·001 per cent. aniline green. It is again washed for from twenty-four to forty minutes with distilled, weakly acidulated, water, in order to decolorize the elements of the tissue. After once more washing in distilled water, the preparation is placed for some minutes in a weak solution of picro-carmine. After being once more washed, the preparation is dehydrated by absolute alcohol, or simply by drying, and finally, if necessary, is cleared with oil of cloves and put up in Canada-balsam.

In order to study bacteriads in the interior of the tissues, it is best to harden the tissues by placing them for at least from one to two days in absolute, or at least 90° to 95° alcohol. For staining the bacteriads the colours already known to us come into use. In preparations stained with gentiana-violet or with methyl-violet, the tissues are completely decolorized with strong alcohol in which is a trace of potash, while the bacteriads retain the colour. A like effect can be attained by laying the preparation for at most a half-minute in picric acid, whereby the tissue takes at once a yellow coloration. After decolorizing the tissue in alcohol this can be again stained with iodine-green, methyl-green, eosin, magdala, acid-fuchsine, and other stains, which are not taken up by the bacteriads.[14] Good double-staining is also attained by gentiana-violet and picro-carmine.[15] The best means for staining bacteriads in the interior of the tissues is, however, usually a solution of gentiana-violet in aniline water, and a solution of potassium-iodide iodine.[16] The aniline water is prepared in the way given upon page 234, and dry gentiana-violet is dissolved in it to saturation, or 5 parts of a saturated alcoholic solution of

gentiana-violet are added to 100 parts of this water. This is filtered before each time of use. The solution can be kept for months. The sections are transferred from absolute alcohol, for some minutes, into the staining fluid, then for from one to three minutes into dilute potassium-iodide iodine solution (1 part iodine, 2 parts potassium-iodide, and 300 parts distilled water), then into absolute alcohol. In this the sections must be decolorized. They are then cleared in oil of cloves, and embedded in Canada-balsam dissolved in xylol. The tissue now appears decolorized, the bacteriads stained dark blue. The *bacilli* of typhus, also the *cocci* in many cases of pneumonia, are decolorized by this process, and are distinguished thus from most other *bacilli*. Treatment for a very short time with a weak solution of Vesuvin before placing in oil of cloves gives beautiful double coloration, in that the tissue now appears stained brown. Instructive coloration is also obtained with safranin upon sections which were hardened in alcohol or in chromic acid.[17] Equal parts of a concentrated watery and a concentrated alcoholic solution of safranin are mixed together, and the sections allowed to lie in this for half an hour, then washed a little in water, and for some minutes in absolute alcohol, transferred to oil of turpentine, and put up in Canada-balsam.

In order to find bacteriads in tissues, after they have been completely stained, Abbe's apparatus [or other condensor] can be used with great advantage, and in a special fashion.[18] After focussing the preparation the diaphragm is completely removed, so that the cone of illumination filling the entire objective comes into use. Thereby the figures of all uncoloured parts, which are only distinguishable by differences in their refractive indices, more or less completely disappear, while the coloured light-absorbing bodies remain visible. We distinguish this method as "isolation of the coloured images."

After we have thus made ourselves acquainted with the different developmental forms and methods of research, we will now point out the methods of **culture**, which come into use for breeding bacteriads; we will breed a definite bacteriad, and follow out moreover its entire development. For this purpose we soak dry hay[19] in the smallest possible quantity of spring water, and let the infusion stand for four hours in a warm chamber at a constant temperature of 36° C. (= about 96° F.). Then pour off the extract without filtering, and if it is too concentrated dilute it, so as to be more safe, to a specific gravity of 1 004. Now place the fluid

in a flask holding at least 500 ccm. The flask is stopped with cotton-wool, and the fluid then boiled very gently for an hour. Then let the temperature sink to, and remain at 36° C. In the course of a day or a day and a half, a delicate grey skin will have formed on the surface of the fluid; this consists of the *zooglœa* stage of *Bacillus subtilis*, the bacteriad of hay. We have made use of the power possessed by the spores of this bacteriad, of resisting boiling heat for a long time, in order to obtain a pure culture of them. Bacteriads in general are distinguished by their power of resisting high temperatures, the bacteriad of hay stands, however, foremost in this respect. Of the pellicle obtained as above we now transfer a little, with a suitable quantity of the fluid to an object-slide, and examine the object with the strongest magnification which we have at command. We find the pellicle

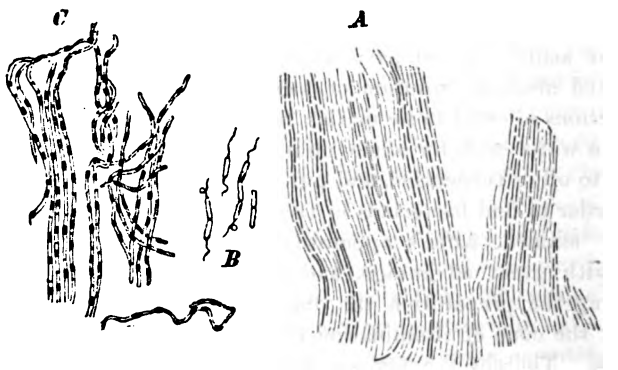


Fig. 85.—*Bacillus subtilis*. *A*, the pellicle (× 500); *B*, "swarming" rodlets (× 1000); *C*, spore formation (× 800).

formed of long, segmented, wavy threads, running parallel to one another. The threads remain for the most part in their position, because they are held together by an invisible jelly (Fig. 85 *A*). The threads consist of cylindrical rodlets of various length, in general however, twice or thrice as long as broad. The substance of the threads appears homogeneous, colourless, pretty strongly refractive. Even with the strongest magnification no other structure is recognisable. With chlorzinc iodine the rodlets are stained throughout, and very clearly, a brownish yellow. The figures are thus obtained better than with the other solutions of iodine. The segments of the threads appear thereby in general shorter than in the fresh state, because now all the limits are

visible. In order sharply to differentiate the rodlets, we can stain them, according to the methods already known to us, with fuchsine, methyl-violet, gentiana-violet, or Vesuvin, and then keep them as permanent preparations in Canada-balsam or in dammar.

Picro-nigrosine can also be used with advantage for fixing and staining the preparation.

If we focus upon a particular spot in the pellicle with a magnification of about 1000, we can observe the **division** (segmentation) of the rodlets direct.[20] It is best to draw the piece of the thread in question at short intervals with the camera, and compare the drawings, so as to show the changes which have taken place. If abundant food-stuff is still in the fluid, the individual rodlets divide every half-an-hour to an hour and a half. The higher the temperature of the room, the more rapid the division. The rodlets increase in length without becoming thinner; when they have attained, however, a definite size, a dark partition-wall appears across their middle. This process of division explains the arrangement of the rodlets and threads; it explains also the wavy course of the threads, which grow at all points by intercalary growth, and if the ends cannot become further removed, the thread must become laterally contorted. For this reason, the whole pellicle shows a wrinkling visible to the naked eye. We next transfer a fragment of the pellicle into a moist chamber, in order to examine it in a suspended drop. For this purpose we will use the simplest possible moist chamber, to wit a small frame of pasteboard. Such a frame is cut out of tolerably thick pasteboard, its inner aperture being somewhat smaller than the size of the cover-glass we propose to use, while its outer diameter does not exceed the width of the object-slide. This frame is soaked in water till it is completely saturated, and then laid upon an object-slide.* On the middle of a cover-glass is placed a drop, spread flat, of the culture fluid, into which the object for investigation is transferred. The cover-glass is turned rapidly upside down, with the drop hanging below, and laid upon the pasteboard frame. If the observation is to be long and continuous, a drop of water is from time to time placed upon the frame, so that it shall not become dry. If the observation is interrupted, the preparation can

* After and before using the pasteboard culture-cell it is desirable to place it for a few minutes in alcohol, so as to kill any organisms which may adhere to it, or the culture may be vitiated. This applies equally strongly to other cultures. [Ed.]

be protected from evaporation in a large moist chamber. In order to again find a definite spot in the preparation, the object-slide must be brought back again into its original position, for which its outline can be drawn with a sharply pointed pencil upon the stage. It is still better in this and similar cases, to cut a cross on the stage by means of a sharp instrument, right and left of the central aperture. Then, when the object-slide is in the required position, similar crosses can be made upon it with one of the sharply pointed colour pencils mentioned in the Introduction or with a writing diamond. It is then easy later on to replace the object-slide thus marked in exactly the same position.* If the food materials of the drop are exhausted, the vegetative segmentation or bipartition is arrested and the spore formation at once begins. After the lapse of from six to eight hours there can be seen in the threads, at thereabouts equal distances, ellipsoid, strongly refractive spores (Fig. 85 *C*). Elsewhere the threads appear empty; only the colourless sheaths unite the spores. At some places in the preparation, one is certain to find spores still in course of formation. They appear in the form of collections of more refractive material situated most usually towards the middle of the rodlet. The aggregation becomes continually stronger, while the rodlet becomes emptied, and at last the formation of the spore is complete. If the culture is allowed to continue some hours longer, the sheaths of the rodlets will have become indistinct, and after the lapse of about a day the spores appear free, and sunk to the bottom of the drop. In contradistinction to the rodlets, the spores hardly, or not at all, stain with gentiana violet and the other stains we have recommended, with the exception of the carbolized fuchsine and alcohol solution given on page 235, which, especially when warmed, stains the spores very deeply. The spores germinate very easily if they are transferred to fresh nutrient fluid; more slowly at the temperature of the room, quicker at 30° C. [=86° F.]. It is best to boil them for five minutes, and cool them slowly. Then in about two to three hours we shall see the commencement of germination.[21] The spore-membrane is opened on one side, the minute germ begins to protrude here, and elongates gradually

* For the purpose of keeping a particular spot under observation for several successive days, *i.e.*, without removal from the stage of the microscope, one end of a few strands of loose wick can be inserted between the layers of the pasteboard, while the other end can dip into a vessel of distilled water. The water sucked up by the wick will keep the pasteboard moist. [Ed.]

into a rodlet. Its hinder end remains inserted in the spore-case. About twelve hours elapse before the rodlet divides for the first time. In the meantime the preparation will show all stages of germination. As a rule, the germinated rodlets at once set up movement, they enter into the "swarming" or "roving" stage. Such a swarming rodlet still carries about at its hinder end its spore-case. The number of the "swarmers" becomes by successive divisions continually greater, and they fill the entire fluid before the beginning of the formation of the pellicle. Then the swarmers collect on the surface of the fluid, come to rest there, and produce the pellicle. The rodlets are of unequal length, and consist of a varying number of segments (Fig. 85 *B*). Their movement is serpentine. We allow the fluid containing the swarmers to dry upon the cover-glass, and stain them then by one of the methods given upon pp. 234–5.[22] The swarmers have a cilium at each end, the identification of which is not easy.[23] They are most readily made visible if the dry cover-glass preparation is stained with watery extract of logwood. In order to fix the stain, the cover-glass can be laid for a time in 0·5 per cent. solution of chromic acid. The preparation is washed in water, and either observed direct in this, or dried, cleared in cedar oil, and enclosed in Canada balsam.

Let us now select for examination *Bacterium Termo*, the constant accompaniment of numerous processes of decay. We may reckon pretty well with certainty to find swarmers of *B. Termo* in water in which peas and beans have been left to rot. We readily prepare a suitable culture if we take a drop of this fluid and transfer it to a proper nutrient solution. For such, Cohn's "normal solution" can be recommended; this consists of 1 gr. acid phosphate of lime, 1 gr. sulphate of magnesia, 2 grs. neutral acetate of ammonia, and 1 gr. calcium-chloride dissolved in 200 grs. distilled water. By repeated transfer of infected drops into new culture fluid, a pure culture will at length be obtained. It is characteristic for *B. Termo* that in a few days the culture fluid becomes milkily turbid, and forms then a greenish pellicle. Microscopical investigation shows rod-like cells, of about 0·0015 mm. long, and from $\frac{1}{2}$ to $\frac{1}{3}$ that in breadth, in course of active bipartition, and thence united in pairs, but scarcely in large rows. The movement is peculiarly jerky. Motionless individuals fill the zooglœa, which ultimately forms a greenish slimy skin or clots on the surface of the fluid. Spore-formation has not yet been observed. If we examine swarm-

ing bacteria in a drop of fluid under a cover-glass, it will be seen that the movement soon ceases. For the observation of this phenomenon the swarmers of *B. Termo* are very well suited. After obtaining an active pure culture, as described above, a drop of the fluid containing the swarmers is observed under a cover glass; the movement is soon seen to cease. It is continued longest around any enclosed air bubbles, and at the edges of the cover-glass. Round the edge a thick layer of swarmers will soon have collected, cutting off all entrance of air. Ultimately all the swarmers come to rest. If, however, in making the preparation we have enclosed in it a green algal thread, the movement of the swarmers continues around this thread so long as it is subject to the influence of light. They collect in considerable number round the thread, and if this contains chromatophores confined to special parts, these parts are sought out by the bacteria. The oxygen given out by the chromatophores acts as a stimulus, which causes the movement of the bacteria, and determines even the direction of the movement. For example, in *Spirogyra* the aggregated bacteria follow the green band. If the preparation is placed in darkness, the movement ceases also around the green cells; and it recommences immediately the thread is again exposed to the action of light, whereby it begins to assimilate and to give off oxygen. The swarming stage of bacteria is therefore a very suitable reagent for oxygen, and it has been made use of in order to measure the strength of the assimilation of carbon in the different parts of the spectrum, and so in some degree to measure the relative values for this purpose of the various rays which constitute white light. Special micro-spectral objectives can be obtained for the purpose of throwing a small spectrum upon the object slide under the microscope; or, if such a micro-spectroscope is not at our disposal, we can obtain an incomplete idea of the energy of the assimilation of carbon under the influence of different rays by allowing the light to pass through coloured glass or coloured fluids the spectroscopic character of which we had previously determined.

Breeding experiments with bacteriads are chiefly carried on in Florence or conical flasks, or in test-tubes.[24] Many cultures are carried on direct upon the object-slide. Object-slides, vessels, and all the utensils to be used must be *sterilized*. This is effected by passing them quickly through a gas or spirit flame, or laying them before the beginning of the experiment in absolute alcohol, which quickly evaporates after removal. The particular nutrient fluids

for the cultures are boiled in the vessels which are to be used, and which must be closed with a cotton-wool stopper. In general it is desirable to boil the nutrient fluids for a short time on each of several successive days. In this way all the bacteriads which have in the meantime germinated, and which then bear a high temperature far less than do the spores, are killed. It is assumed that after five days all the spores are killed. For greater certainty the nutrient fluid can be allowed to stand another day, before it is used for the inoculation; if it remain clear, it is assumed that it is sterilized. That boiling for an hour does not always serve for killing the spores, we can see in the culture of *Bacillus subtilis*. The infection of the cultures arises usually, not from the air, but from incompletely sterilized vessels. The danger of infection from the air in the temporary opening of the vessels for the purpose of sowing ("inoculation") is far less great than that which arises from incompletely sterilized vessels.[25]

To obtain pure material for inoculation in cultures on a large scale, various methods can be followed:—

1. *The method of fractional culture.*[26] This is based upon the experimental fact that of several kinds of bacteriads growing in the nutrient fluid, one ultimately gets the upper hand. If now from a culture which has progressed thus far a little is transferred into a second solution, free of fungi, and after a similar length of time from this into a third, and so on, there is a chance of ultimately obtaining a perfectly pure culture, that form always remaining last which, under the given conditions, multiplies most quickly.

2. *The Method of Dilution.*[27] When the bacteriad which is to be bred preponderates in the fluid, this method gives usually very good results. The fluid containing the bacteriad is diluted with water free of fungi, until by casual estimation only one bacteriad comes into a drop of the fluid. If the form to be bred distinctly preponderates, and a series of vessels containing the nutrient fluid are inoculated each with a drop of the fungus-containing solution, all the probabilities are that in the majority of the vessels pure cultures will be obtained. [See note[27*].] Whether a culture in a nutrient fluid is pure can in general be determined even macroscopically, by the fluid being uniformly turbid, or showing uniform formation of skin on the surface, or uniform formation of clouds at the bottom, or uniform coloration, or uniform formation of jelly. The purity of a culture is likewise

assumed in which strong fermentation or intense putrescence results.[28]

3. *Gelatine Culture.*[29] This method gives the best results, and has led to the greatest progress in our knowledge of bacteria. In it the nutrient fluid is mixed with gelatine, with agar-agar,* or with blood serum. Most commonly used is a mixture of infusion of peptone and gelatine, in which the gelatine forms 5 per cent. 0·50 grm. gelatine is soaked and boiled in 500 ccm. water. Half a kilo. of chopped meat is allowed to stand cold in 500 ccm. water for 24 hours, then the meat-infusion, obtained by pressing the meat, is boiled, filtered through fine gauze, and mixed with the gelatine; add 10 grm. peptone and 1 grm. common salt, neutralize it with carbonate of potash, or with carbonate of soda or phosphate of soda, and filter through filter paper.† Put into a test-tube 10 to 15 ccm. of this nutrient gelatine, close it with a plug of cotton wool, and sterilize it by continuous boiling for many hours, or better by boiling for half an hour on each of several successive days. In many cases it is recommended during the final cooling of the nutrient gelatine to bring the test-tube into a somewhat inclined position, whereby the free upper surface of the fluid is enlarged. According to need, the quantity of nutrient gelatine can be reduced to 2·5 per cent. or raised to 10 per cent. In similar manner to this meat-infusion peptone gelatine, can also be prepared hay-infusion gelatine, wheat-infusion gelatine, aqueous humour gelatine, meat-extract peptone gelatine, meat-infusion peptone gelatine, with 1 per cent. cane or grape sugar, etc. In case the culture should be maintained at incubation temperature, it is preferable to add agar-agar or blood serum to the nutrient fluids instead of gelatine. Such a nutrient basis remains solid at incubation temperature, while gelatine nutrient basis becomes fluid. It is prepared by adding 1 per cent. agar-agar to the nutrient fluid. The method of preparation of cooled blood-serum is more complicated. The blood of the killed animal is drawn immediately out of the wound into a pretty tall vessel provided with a glass stopper and previously sterilized. This vessel is filled right up to the brim, and placed for 24–30 hours in a refrigerator or ice-bath, until a copious layer of entirely transparent amber-yellow

* Agar-agar, or Bengal isinglass, is a species of dried seaweed from Singapore, consisting of small, colourless, transparent strips; is almost completely soluble in water, and forms a thick, tasteless, and odourless jelly.—[Ed.]

† One-tenth of these proportions would fully suffice.—[Ed.]

coloured serum is formed over the cake of blood. By means of a pipette a test-tube is filled with the serum, and stopped with a cotton-wool plug. The plug has been previously heated for an hour in a warm bath of 150° to 160° C., and so sterilized. The blood-serum should now be warmed in the open water-bath, on five successive days, for one hour each day, to a temperature of 58° C. On the last of these days the temperature should be allowed to rise, from half to one hour, to 65° C., by which the blood-serum "sets." Sheep's serum sets the most quickly, calf serum the most slowly. The coagulated serum must be completely clear and pellucid; if it is not perfectly sterilized it becomes cloudy immediately.* It can be used by itself or be added as a "setting" constituent of the nutrient fluid. The solid nutrient basis can also be used with good results for microscope slide culture. While the sterilized nutrient gelatine, agar-agar, or blood-serum respectively are still fluid, a small quantity of either can be poured on a sterilized object-slide, so that the layer of it after setting is about 2 mm. ($\frac{1}{12}$ inch) thick. After the subsequent inoculation this object-slide is placed under a bell-jar closed with water, or in a case made of plaster of Paris. A case made entirely of plaster of Paris, with a plaster of Paris cover, is very suitable as a moist chamber for the cultivation of fungi and bacteria, which do not need the light, because the moisture is very uniformly distributed in it, and no drops of water fall down from above on to the preparation.[30] Instead of inoculating on the object-slide, this can be done with the nutrient gelatine while still in the test-tube, warmed up to about 25° C. (=77° F.), and so made fluid, with which the inoculating material is uniformly mixed, and which is then poured on the object-slide. If different organisms are represented in the inoculating material, they now form on the object-slide separate colonies, each of which usually represents for itself a pure culture. The purity of individual colonies can be proved directly under the microscope; and in that way pure material can be selected from them for future inoculations. The macroscopical appearance of the colonies is moreover often characteristic, and can lead to the recognition of forms which are otherwise difficult to distinguish microscopically. Thus the form of the colonies, their coloration, the circumstance whether the nutrient body becomes fluid or not, whether the colonies themselves ultimately

* All these culture fluids are to be had, ready prepared and sterilized, from Dr. Hermann Rohrbeck, in Berlin, Friedrichstrasse, 100.

stain, serve as good indications of the nature of the bacteriads. The inoculation of a nutrient fluid, or of a solid nutrient body, is effected by means of a needle which has just been heated red-hot, but is already quite cool, or with a platinum wire just heated. For this purpose the solid nutrient body on the object-slide is scratched with the wire or needle after it has been dipped in the bacterial fluid. If the solid nutrient body is in the interior of a test-tube, the needle or wire is stuck in to the depth of from $\frac{1}{4}$ to $\frac{3}{4}$ inch. The mode of development in nutrient bodies inside the test-tube is also characteristic, and often, like to the characteristic signs in cultures on the object-slide, permits macroscopic distinctions of separate forms.

Slices of boiled potatoes are especially suitable as a solid opaque nutrient basis for bacteria. The surface of the tubers is first made free from spores by careful washing, and then exposure for about one hour to the action of a 3 per cent. solution of corrosive sublimate. The tuber is then washed in sterilized water, and boiled so as to make sterilization certain. Only those tubers must be used which do not crack. In cutting the potatoes the necessary precautions must be taken, and the hands also be disinfected with 1 per cent. solution of sublimate. The slices of potato are then placed under sterilized vessels. If the dishes are not completely sterilized, we shall find upon them the so-called potato bacillus, forming a white, then grey, and finally brown wrinkled layer. This bacteriad is actively motile, and is very disposed to the formation of spores, which fill well-nigh the whole mother-cell. It has been more recently proposed to sterilize the potato disks themselves. For this purpose a potato tuber is peeled, then washed and cut up into slices about 1 cm. thick, which are laid singly in small covered glass dishes of corresponding size, and previously sterilized. The dishes are now laid for from three quarters of an hour to an hour in a steam chamber, by which the potato disks are also sterilized. These potato disks can then be preserved unchanged for months.

If it is desired to follow the processes of development of a form directly under the microscope, it is done with the aid of small moist chambers. For pure cultures lasting a longer time the pasteboard chamber previously referred to is not sufficient. For such purposes a chamber made out of a glass ring is recommended.[31] Such a glass ring, about $\frac{1}{5}$ inch thick (high) is broken off from a glass tube of suitable diameter. The glass ring is flattened on

both of its ends (sides) on a whetstone, and fixed upon the object-slide with Canada-balsam. A round cover-glass of suitable size serves as a cover. The thinnest possible layer of the gelatine, agar-agar, or blood-serum nutrient body is placed in the middle of the cover-glass, and this layer is afterwards inoculated. The cover-glass is fixed on the glass chamber by means of oil-drops run round its edge. A thin layer of water at the bottom of the glass chamber secures the necessary moisture. Such a moist chamber can be converted into a gas chamber when the glass ring has two lateral openings into which are melted or stuck glass tubes which serve for the introduction and removal of gases. Another moist chamber, likewise to be recommended,[32] consists in an object-slide with a flat central round or quadrangular hollow, which is surrounded by another narrow groove (or channel) cut still more deeply. This groove is filled with water. The cover-glass used must be of sufficient size to completely cover up this external groove, and project all round on to the unhollowed portion of the object-slide. For cultures at constant and more elevated temperatures, double-walled propagating chambers, with suitable warming appliances, will be needed.*

NOTES TO CHAPTER XXI.

[1] For the statements here following compare Zopf, *die Spaltpilze*; and De Bary, *Vergl. Morph. u. Biol. der Pilze, Mycetoz. und Bacterien*, p. 490; in both these works the general literature is given. For the staining methods I adhere chiefly to Hoyer, *Gazeta lekarska*, 1884.

[2] According to Errera, *Bull. de la Soc. Belge de Micr.*, tom. X., No. 11.

[3] *Zeitschr. für wiss. Mikros.*, Bd. I., p. 411.

[4] According to Brun, taken from Fol, in *Lehrt. d. vergl. mikr. Anat.*, p. 37.

[6] Engler, *Bericht der Commission zur Erf. d. deut. Meere*, 1881; Zopf, *die Spaltpilze*, pp. 13, 75, et seq., where the other literature is quoted.

[8] Compare Cohn, *Beiträge zur Biologie*, Bd. I., p. 125.

[9] Compare the literature on this subject in Zopf, *die Spaltpilze*, 1883.

[10] Zopf, *l.c.*, p. 5. [See also W. B. Grove, *Bacteria and Yeast Fungi*, 1884.]

[11] From R. Koch, *Berliner, klinische Wochenschrift*, 1882, p. 221.

* Such an apparatus can be obtained from Dr. Robert Muencke, in Berlin, Louisenstrasse, 58, or from Dr. Hermann Rohrbeck, Berlin, Friedrichstrasse, 100, at an expense of from 25 to 50 M. The propagating chambers of d'Arsonval, which cost however of Dr. Muencke from 72 to 108 M., or of Dr. Rohrbeck 28 to 130 M., are to be specially recommended.

[12] Compare on this point C. Friedländer, *Mikr. Technik*, II., edit., p. 53.

[12a] Neelson's method, according to Hoyer.

[13] According to Soubbotine, *Arch. de Phys. norm. et path.*, Tom. XIII., 1881, p. 477.

[14] According to Hoyer, *l.c.*

[15] Weigert, *Virchow's Archiv*, Bd. LXXXIV., p. 201; Firket in Bizzozero's French translation of the *Manuel de micr. clin.*, p. 314.

[16] Gram, *Fortschr. d. Med.* 1884, p. 185.

[17] Victor Babes, *Archiv. fur mikr. Anat.*, Bd. XXII., pp. 359 and 361.

[18] Introduced by R. Koch; *Unters. über Aet. d. Wundinfectionskrankheiten*, Leipzig, 1878.

[19] According to a method recommended by Roberts and Buchner; compare Zopf, *die Spaltpilze*, p. 57, to which work I have in general referred as a source for the other literature.

[20] Compare Brefeld, *Schimmelpilze*, Heft IV., p. 38.

[21] Brefeld, *l.c.*, p. 43.

[22] Cf. Koch, in Cohn's *Beitrage z. Biolog.*, Bd. II., p. 402.

[23] Brefeld, *l.c.*, p. 40.

[24] Buchner, in Naegeli's *Unters. üb. niedr. Pilze*, p. 192, where are representations of the special forms of glasses used for culture experiments.

[25] Buchner, *Stzber. d. kongl. bair. Akad. der Wissensch.*, 1880, p. 381, and in Naegeli's *Unters.*, as above, p. 159.

[26] Employed by Klebs; *Archiv. f. exper. Path.*, Bd. I., p. 46; for the rest I have again had recourse to Zopf, *Spaltpilze*, pp. 43 ff.

[27] From Naegeli, *Stzber. d. kgl. bair. Ak. d. Wiss.*, 1880, p. 410, and *Unters. über niedr. Pilze*, p. 13; Buchner, *Stzber. d. kgl. bair. Akad. d. Wissensch.*, 1880, p. 374, and in Naegeli's *Unters. über niedr. Pilze*, p. 146.

[27a] According to H. Fol and P. L. Dunant (*Arch. des sc. phys. et nat. de Genève*, tom. XIII., 1885, p. 116), this same method can be employed to determine the number of Bacteria contained in a given quantity of a fluid. This fluid is diluted up to a certain point with sterilized water; then a portion is taken and added to a known quantity of nutrient fluid, and this mixture is divided equally amongst a number of glasses. The number of glasses which remain sterile, compared with the total number, and with the quantity of fluid taken, enables us to calculate the number of Bacteria in the original fluid. If all the glasses contain Bacteria, the fluid employed has not been sufficiently diluted.]

[28] According to Zopf, *l.c.*, p. 44.

[29] Introduced by Brefeld; compare *Schimmelpilze*, Heft I., p. 15. Completed by R. Koch. Compare R. Koch, *Zur Untersuch. pathol. Organismen*, extracted from *Kaiserl. Gesundheitsamte*, 1881, p. 18, and numerous other researches in the same.

[30] Bainier, *Annal. des Sc. Nat. Bot.*, Series VI., tom. XV., p. 346.

[31] According to Van Tieghem and Le Monnier, *Annales des Sciences Naturales, Botanique*, V. ser., tom. XVII., p. 263.

[32] Dippel, *Das Mikroskop*, 2nd edit., p. 662; *Grundzüge der allg. Mikr.* p. 295.

[The best work upon Bacteria and Bacteria culture in the English language is Crookshank's *Manual of Bacteriology*. London: Lewis.]

[*Note to page* 222.]

[a] The protoplasm of bacteria, like other protoplasm, colours brownish-yellow with iodine, and may be either homogeneous or granular. In general it is naturally colourless, in a few cases is green from the presence of a chlorophyll-like colouring matter (*Bacillus viridis*) or bright-red (*Beggiatoa roseo-persicina*). It is quite possible that on our vegetable disks some coloured form may make its appearance.

[*Note to page* 223.]

[b] Instead of using an infusion for the purpose of rearing bacteria, we can, as noted above, use a solid substratum of an organic nature. Very favourable for the purpose are slices of potato or of the white of a hard-boiled egg. If these are kept in a warm place, and covered with a bell-jar, so as to retain a moist atmosphere, or according to one of the methods hereafter to be detailed, various patches of coccus bacteria, often highly coloured, will make their appearance, amongst them probably being the blood-red *Micrococcus prodigiosus*. These patches enlarge, and at length join into a kind of skin. On the potato they usually take the form of a white, later on grey, and finally brown, wrinkled skin.

CHAPTER XXII.

THE REPRODUCTION OF ALGÆ.

Material Wanted.

Spirogyra in conjugation. Fresh.
Cladophora glomerata, taken from quickly-flowing water. Fresh.
Vaucheria sessilis. Fresh. Also the terrestrial form of the same, with the sexual organs.*
Fucus platycarpus. Fresh, and in alcohol.
Fucus vesiculosus. Fresh, and in alcohol.
Chara fragilis, and *Nitella* sp. Fresh.

Now that we have obtained information in the general sphere of morphological investigation amongst higher as well as lower vegetable organisms, it will be our task to make ourselves acquainted with the most important of those problems which the special morphology of microscopical investigation provides. In this we shall take just the opposite way to that we have hitherto followed, and slowly mount from the simplest groups of organisms to those most highly organized. We have already made a commencement in our last chapter upon the Bacteria, to the entire cycle of development of which we have directed our attention. In the whole of this life-cycle there was no indication of separate sexuality. The process of multiplication was vegetative, or asexual. Organisms of somewhat higher grade, however, show both of these processes, vegetative or asexual multiplication, on the one hand of entire organisms, or on the other hand of the constituent cells of those organisms, and the commencement of the life-cycle of new individuals through processes of more or less complicated sexuality. We will now continue with the examination of the asexual and sexual processes amongst the Algæ.

Opportunity is not rare for examining the various species of *Spirogyra* in process of **Conjugation.**[1] This is recognisable out of doors by the crinkled look [rather yellowish] and hanging together

* All these Algæ can probably be obtained from T. Bolton, Newhall Street, Birmingham. [Ed.]

of their masses of threads. The process can be easily followed, but the threads must not be directly covered with a cover-glass upon the object-slide; but on the other hand the small pasteboard moist chamber, described on page 174, will serve with advantage, and then the *Spirogyra* is placed in the drop suspended from the cover-glass. Conjugation in most species takes place in ladder-like fashion, *i.e.*, two threads lying alongside one another are united by cross-bridges. The cells put out short blunt projections, which come into contact and fuse with one another. In many cases it can be distinguished, before conjugation, which thread is male and which female, since the cells of this latter swell out into barrel-shape. After the union of the conjugating processes, the contents of the male cell tend first to become rounded off, and finally withdraw on all sides from the cell-wall. They then pass into the conjugating canal, and through the partition walls of the two conjugating processes, which in the meantime had become softened. The female cell had simultaneously rounded, or rounds off on entrance of the male cell. Both cells come into contact, and after a few minutes coalesce. Their contents blend; the chlorophyll bands join together; the two nuclei unite into a single one,[2] this, however, not being visible without the use of staining reagents. The **zygote** [**zygospore**] thus formed begins at once to contract; after the course of an hour its cavity [hitherto filled with cell-sap] has completely disappeared [the cell-sap being expelled]. In this contraction the chlorophyll bands are drawn more into the interior, while the periphery appears composed of colourless frothy protoplasm. The zygote [zygospore] is more or less globular. After the lapse of twenty-four hours, it has again enlarged, acquired a cavity, and taken an ellipsoidal form; the chlorophyll bands are pressed to the periphery, and a clear membrane, with double contour, now covers the zygote.

This process of conjugation we have just studied is characteristic of the entire section of Algæ, collected together as **Conjugatæ**. To this, besides the species of *Spirogyra*, so widely diffused in fresh water, belong also the almost equally widely-spread species of *Zygnema*, recognisable by two **stellate chromatophores** in each cell, and the elegantly formed Desmideæ. Into close connection with these latter we can bring the Diatomaceæ, in which typical conjugation is likewise present.

The genus *Cladophora*, belonging to the **Chlorophyceæ**, the structure of which is already known to us, provides us with a

right favourable object for the study of **swarm-spores**;[3] it is only to be regretted that they are not always inclined to the production of swarm-spores. It is comparatively easy to obtain swarm-spores of the marine forms, which we lay in a large vessel with sea-water; still amongst fresh-water forms, *Cladophora glomerata*, if taken from rapidly-flowing water, and laid about evening in a shallow vessel with a layer of water about $\frac{1}{2}$-inch deep, will be found with swarm-spores usually next day. The formation of these commences at the apex of the branches, and proceeds towards their bases. Hence all stages of development are easily found close together. We look at these in the direction from the base towards the apex of the branch, and commence with a still unchanged cell. The structure of this is already known to us. What is visible without reagents we soon recognise again: the polygonal, closely-crowded chromatophores, which contain small, pale starch-granules, in part also having larger amylum-groups; the plasmic plates which traverse the cavity of the cell, and in part also contain chromatophores. If we pass now gradually from such a cell to such as are being transformed into **sporangia**, first of all a change of the colour of the contents strikes us. With a sufficiently high power we can determine at the same time the absence of the amylum-groups; these have fallen into individual starch-granules, and simultaneously has come about a subdivision of the chromatophores into smaller ones. In the next stage the chromatophores begin to arrange themselves into a net, so that the entire contents of the cell, surrounding a narrower or wider cavity, appear divided into approximately equal polygonal sections. The middle of each of such sections is free of granules, and fixed and stained objects teach us that a nucleus lies there. At the same time the peripheral layer around the whole contents of the cell increases in thickness, and becomes readily visible. It is especially thick at the angles of the cell. At one place, usually in the neighbourhood of the front end of the cell, and in terminal cells occupying the anterior end, is still noticeable a lenticular aggregation of colourless protoplasm. Corresponding with the centre of this aggregation the membrane of the cell swells and bulges out, likewise as the result of the increase in volume due to the swelling, into a papilla-like projection. The next change consists in that the chromatophores withdraw towards the interior of the polygonal sections, and these latter appear bounded towards one another by clear lines. The sections then begin to round off, and

so partially to separate from one another. The peripheral sections now project outwardly as roundish knobs. The colourless peripheral protoplasm takes no part in the differentiation of the chlorophyll-containing contents into individual sections, but is transformed into a colourless mucus, which plays a part in the evacuation of the **swarm-spores** [**zoospores**]. Corresponding with the strong aggregation of colourless protoplasm at what later on is the place of exit, the mass of slime formed is here the largest, and the still connected mass of swarm-spores remains, therefore, at this place, somewhat removed from the swelling cell-wall. In the mulberry-like mass of the swarmers the cylindrical, more strongly or feebly developed cavity is now easy to see. In a sporangium very rich in contents it may be wanting. In general it is, however, present, so that the swarm-spores form a double or triple layer around this inner hollow. The swarmers soon take on a pear-like form. The anterior, colourless, tapering end is easily distinguishable from the rounded chlorophyll-containing posterior end; on the surface of each swarm-spore appears a narrow, reddish-brown fleck, the so-called **eye-spot.** The cell-membrane, at the part corresponding with the papilla, is already so strongly swollen that its outline is difficult to recognise. With continuous observation we shall now soon see the moment arrive when the sallying of the swarm-spores begins. Under the pressure of the contents the swollen substance of the papilla is broken through, and the mass of the swarm-spores strongly pressed forwards. At the same time with the swarm-spores, finely granular masses of contents of the cell-cavity move outwardly. After a while the forward-pressed swarm-spores set up a motion. The contents of the sporangium, decreasing in bulk, withdraw from the cell-wall; apparently the mass of jelly which lies there presses on the cell-contents. If only a few swarm-spores are present in the sporangium, they now begin to move about confusedly, and one after another pass outwards through the papilla. A small number can also remain behind in the sporangium. If the object is examined in a suspended drop, under the influence of light, the swarmers ultimately collect either at the side of the drop turned towards, or at that turned from, the window. These swarmers are not, however, amongst those most sensitive to light; they remain for a longer time scattered in the drop, move about in indefinite directions, and only gradually, while their motile energy diminishes, arrive at the edge of the drop, where they come to rest. They then round off, and surround

themselves with a cell-wall. With a little potassium-iodide-iodine, these swarm-spores can be very well fixed (Fig. 86). We recognise now two cilia upon each (or, with the species of *Cladophora*, even four), which arise from a small projection from the anterior end of the swarm-spore. The swarm-spores move with the ciliate end forwards. In swarm-spores lying in a favourable position, the small nucleus is thoroughly recognisable, after treatment with iodine, lying in the anterior colourless end (compare the figure); the nucleolus usually stains very sharply.

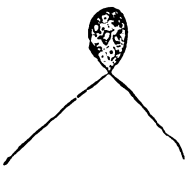


FIG. 86. — *Cladophora glomerata*. A swarm-spore fixed with potassium-iodide-iodine. On the right hand is seen the eye-spot; in the anterior colourless portion the nucleus is to be seen (× 540).

These swarm-spores observed by us are asexual, but in *Cladophora* still other, smaller, sexually differentiated swarmers, or **Gametes**, are produced. These conjugate with one another, but have hitherto been observed only in the marine forms.[4]

From amongst the **Siphoneæ** we select for examination the widely-spread *Vaucheria sessilis*, in order to study the formation of its swarm-spores (zoospores) and sexual organs. If we have collected strong specimens of this alga in standing, or better still in flowing, water, and afterwards placed then in a shallow vessel with fresh water poured over them, we can pretty certainly reckon on numerous swarm-spores by next morning. These are being turned out the whole forenoon, so that we can easily find all the desired stages.[5] If we examine the culture with a lens of considerable focal distance, we can easily recognise the first formation of the sporangia by the dark colour of the ends of the threads. If now we seize with the forceps, at their point of junction, a group of threads which appear to be in the desired condition, and transfer them, without allowing them to be bent, to an object-slide, we may study upon them directly the further processes of development. Moreover, these often go on undisturbed under the cover-glass, if only the object is protected from the pressure of the cover-glass by minute fragments of elder-pith, or horse-hairs, placed under its edges. If a sporangium has been formed out of the end of a branch, contents rich in chlorophyll collect in this, and at the same time the end of the twig begins to swell into a club. The hollow in the club is narrowed (Fig. 85, *A*), and is soon separated off in the upper part of it as a

spherical vacuole. The sporangium is then cut off by a partition wall, in the formation of which the chlorophyll-containing contents of the young sporangium and of the rest of the sac temporarily separate from one another, so that we can see them separated by a clear interspace (Fig. 87, *B*). Around the contents of the sporangium is now formed a clear border (*E*), which soon shows radial structure. This border consists of colourless protoplasm, the radial structure arises from the elongated, radially-arranged nuclei, which are here collected (*F*, *G*). These nuclei show up clearly only after treatment with suitable reagents, and are only visible with strong magnification.[6] The swarm-spore of *Vaucheria* is therefore **multinuclear**. When the swarm-spore is fully formed, it is at once evacuated. The apex of the sporangium ruptures with a jerk, and at the same moment the anterior part of the swarm-spore flows out of the opening, and simultaneously begins to rotate upon its axis. The swarm-spore has to squeeze through the opening. Its birth lasts usually somewhat over a minute. A swelling substance formed in the sporangium helps to expel the swarm-spore. In many cases, though comparatively seldom, the anterior end of the swarm-spore twists off from the hinder part, still in the sporangium; the anterior part then hastens to form a complete, but correspondingly small, swarm-spore, and the posterior part forms a second swarm-spore. This is only possible by virtue of the multinuclear character of these

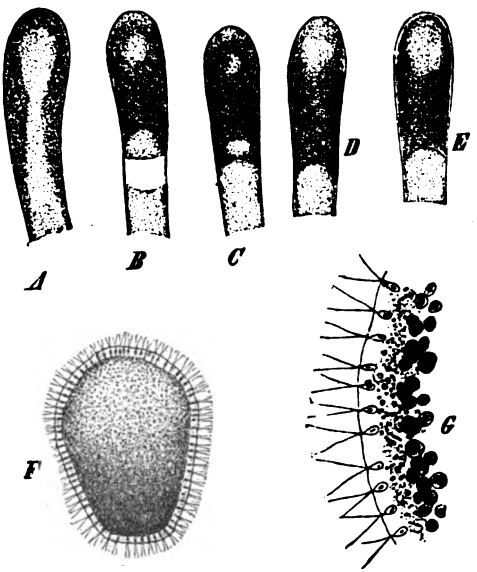


FIG. 87.—*Vaucheria sessilis*. *A* and *B*, formation of the sporangium; *C-E*, formation of the swarm-spore out of the contents of the sporangium; *F*, a swarm-spore set free; *G*, a portion of the outer, colourless plasmic layer ["ectoplasma"], taken from the anterior end of the swarm-spore. *A*–*E* (× 95); *F* (× 205); G (× 450).

swarm-spores, in that each half contains the nuclei necessary to its existence. The movement of the swarm-spore lasts about a quarter of an hour; the direction of the movement is not influenced by the direction of the rays of light falling upon it. The swarm-spore is egg-like in form; in the anterior part it is broader; in this anterior end lies the cell-cavity [vacuole]. Only in the moment when the swarm-spore comes to rest are the cilia visible; they cover the whole body as a short down. In the next moment they are withdrawn into the body of the swarm-spore, which, during this process, shows a wrinkled surface. Afterwards the body is again smooth. During the withdrawal of the cilia it is noticeable that the swarm-spore has already surrounded itself with a very delicate membrane. The spore now rounds off slowly; its colourless border disappears, while the chlorophyll-grains come to the surface; the cell-wall rapidly becomes thicker.

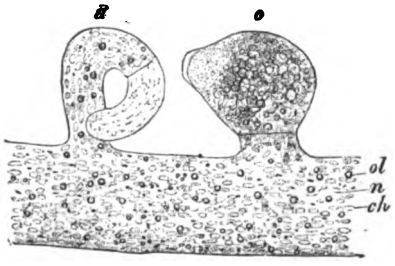


FIG. 88.—*Vaucheria sessilis*. Portion of the thallus with sexual organs. *o*, oogonium; *a*, antheridium; *ch*, chromatophores; *ol*, oil-drops. The nuclei *n* are inserted, although they are only visible after suitable staining (× 240).

In the terrestrial form of *Vaucheria sessilis*, Vauch., the sexual organs are found very easily. The species is recognisable in that the female organs, the **oogonia**, are *sessile* (*i.e.*, unstalked) upon the filamentous thallus, and that the male organs, the **antheridia**, on the other hand, arise as a direct continuation upon a short, horn-like curved branch, arising immediately from the filamentous thallus. An antheridium and an oogonium arise usually in a pair quite close together; not infrequently, however, an antheridium can be seen between two oogonia. This species of *Vaucheria* is chosen for examination, and not that which is met with quite as commonly upon damp earth, in which oogonia and antheridia are seated upon a common lateral branch, which is ended by the oogonium. This last species, *Vaucheria terrestris* Lyngb., is little suited for examination. The aquatic *Vaucheria sessilis*, when in culture, forms at first the swarm-spores already described, and tends only after some weeks to produce sexual organs. The **oogonium** is obliquely ovate, thickly filled with plasma containing chlorophyll and oil, separated from the thallus-

thread by a partition wall placed somewhat above its point of insertion. The oogonium is provided with a unilateral, beak-like outgrowth, in which colourless protoplasm is collected. In advanced stages of development of the oogonia this latter occupies the entire upper third of the egg [-cell, or **oosphere**].* If now we observe such an oogonium continuously, we shall see the colourless substance at the beak end put out a papilla-like projection, which rounds off more and more into an independent ball; this separates finally from the contents of the oogonium, and is thrust out into the surrounding water, where it slowly goes to the bottom. Direct observation shows us that in this the membrane of the oogonium at the end of the beak is not perforated, but rather swells into a jelly, and that the issuing plasmic drop is pressed out through the jelly. The remaining contents of the oogonium round off, its colourless apex is the place of fertilization [**receptive spot** of the oosphere, or egg-cell].—The antheridial branch is more or less strongly curved. Its upper third is formed into an **antheridium**, and is cut off by a partition wall (Fig. 88, *a*). In the ripe condition it is distinguished by its colourless contents, while the branch which bears it is rich in chlorophyll-grains. The antheridium usually turns its apex away from the oogonium. In the colourless contents of the antheridium short rodlets, arranged longitudinally, are more or less clearly distinguishable. At the moment when the oogonium presses out a portion of its colourless protoplasmic substance, the antheridium opens at its apex, and evacuates its slimy content. The greater part of this remains in the surrounding water in the form of colourless bubbles, where it slowly disorganizes; a smaller part hastens away in the form of minute glancing **spermatozoids** [**antherozoids**]. These actively swarming spermatozoids soon collect in the mass of jelly at the apex of the oogonium. Individuals press forwards to the colourless receptive-spot of the egg [-cell], and as it were grope around it. In specially favourable cases a fusion of such a spermatozoid with

* Some explanation of the terminology is needed here. The female cell, prior to fertilization, the Author calls the "egg." By others it is known as the "egg-cell" or "oosphere." After fertilization, keeping up the analogy with conjugation, the Author calls it "zygote"; by others this fertilized cell is called "egg" and "oospore." The use of the terms "zygospore" and "oospore" implies the morphological difference between the fertilizing cells in the two cases; the use of the one term "zygote," as common to both, emphasizes the physiological unity of the sexual process, even when more highly evolved than here. [ED.]

the receptive-spot can be determined. After a short time the fertilized egg [or **oospore**], the **zygote**, has surrounded itself with a delicate membrane, which is especially clearly visible at the receptive-spot. In the course of some hours the colourless protoplasm of the receptive-spot is diffused equally in the zygote. Older zygotes are thickly filled with oil-drops, show some brown spots in the interior, and have a firm membrane.

If a spermatozoid in course of movement is fixed with potassium-iodide-iodine, two **cilia**, unequally long, unilaterally inserted, and extended in opposite directions, can be seen attached to it.

The various species of *Fucus*, or brown sea-weeds, found everywhere round our coasts, can be obtained in fructification nearly the whole year round. If they are gathered at high tide, when they are under water, or immediately after the setting in of the ebb, and are sent damp, without any other packing, you are pretty certain to be able to observe the phenomena of fructification even at places far distant from the sea. The parcel ought to be accompanied by a considerable quantity of sea-water. After receipt, a portion of the plants should be hung up free on string, the other part laid in sea-water. In about six hours, after the sexual organs have been emptied, the hanging plants can likewise be laid in sea-water, and after about six hours again taken out and hung up, and thus the evacuation of new sexual products induced. If the plants which immediately on arrival were hung up have not yielded sexual products, they may be expected from those which were laid in water, if these are taken out after about six hours and hung up to dry slowly. In cool weather the plants can stand a journey for several days without injury, and by laying in sea-water periodically can develop normal sexual products for days.

In order to inform ourselves as to the structure of the sexual organs, we will choose in the first place the hemaphrodite species, *Fucus platycarpus*, Thuret. This species is specially distinguished by the production of male and female sexual organs in the same conceptacle. It is further distinguished from *F. vesiculosus*, which it otherwise closely resembles, in that it is always devoid of air-bladders, while such are very general in *F. vesiculosus*, although not always present. Fertile specimens of both these species end their ultimate branchlets with bladder-like swellings. These contain the **conceptacles**. In *F. platycarpus* the swellings are stronger than in *F. vesiculosus*. Cutting sections of these swollen twigs offers some difficulty, on account of the strong tensions of the

tissues, which result in the outer edges being folded inwards. The bladders collapse somewhat, while a part of the enclosed air escapes audibly. The interior of the bladder appears filled with a filamentous network, and partly also with colourless jelly. Cross-sections prepared between elder-pith show us that the tissue of the thallus has the same structure which we have already studied in *F. vesiculosus* (p. 202*a*): outwardly the layer of small polygonal cells of the epidermoid layer, inwards the progressively enlarging cells of the cortex, which elongate more and more, and ultimately pass over into the network of threads which constitutes the pith. The spaces between the threads are filled with jelly and air. The **conceptacles** are pear-shaped hollows in the tissue. A narrow opening, the **osteole**, communicates with the exterior, and through this passes a tuft of delicate hairs. If the section has cut the conceptacle in the middle line, it is easy to get an idea of its structure. It is seen to be surrounded by a sheath consisting of several layers of closely-united, tangentially elongated cells. From the inner cells of the sheath arise numerous radially-arranged structures, growing into the conceptacle, and reducing its cavity to a narrow cylindrical space, which diminishes as we pass outwards. These structures are, in part, **sterile hairs** which remain unbranched. The number of these sterile hairs diminishes towards the upper part of the conceptacle. The cells themselves are elongated many times as long as broad. The hairs close under the osteole, on the other hand, are composed of short segments. It is these hairs which project from the osteole in a tuft. The cell-contents include protoplasm, nucleus, and very small olive-green chromatophores. In structure much like these hairs, are the copiously branched hairs which bear the male-sexual organs, the antheridia. The antheridia are unicellular branches of these hairs, have an elongated ellipsoidal form, and abundant contents. Nuclei are not visible without special means; small chromatophores are numerous. In the ripening, the contents collect into small balls, each with one, rarely two, reddish-brown chromatophores. Between the sterile and the fertile hairs are found other ellipsoidal structures, the female sexual organs, the **oogonia**, which vary in size according to their stage of development, but ultimately attain to very considerable dimensions. The larger ones are coloured yellow-brown by small chromatophores; the abundant contents make them almost opaque, but without difficulty it can be determined that they contain eight eggs, or **oospheres**, with flattened contact surfaces. The smallest

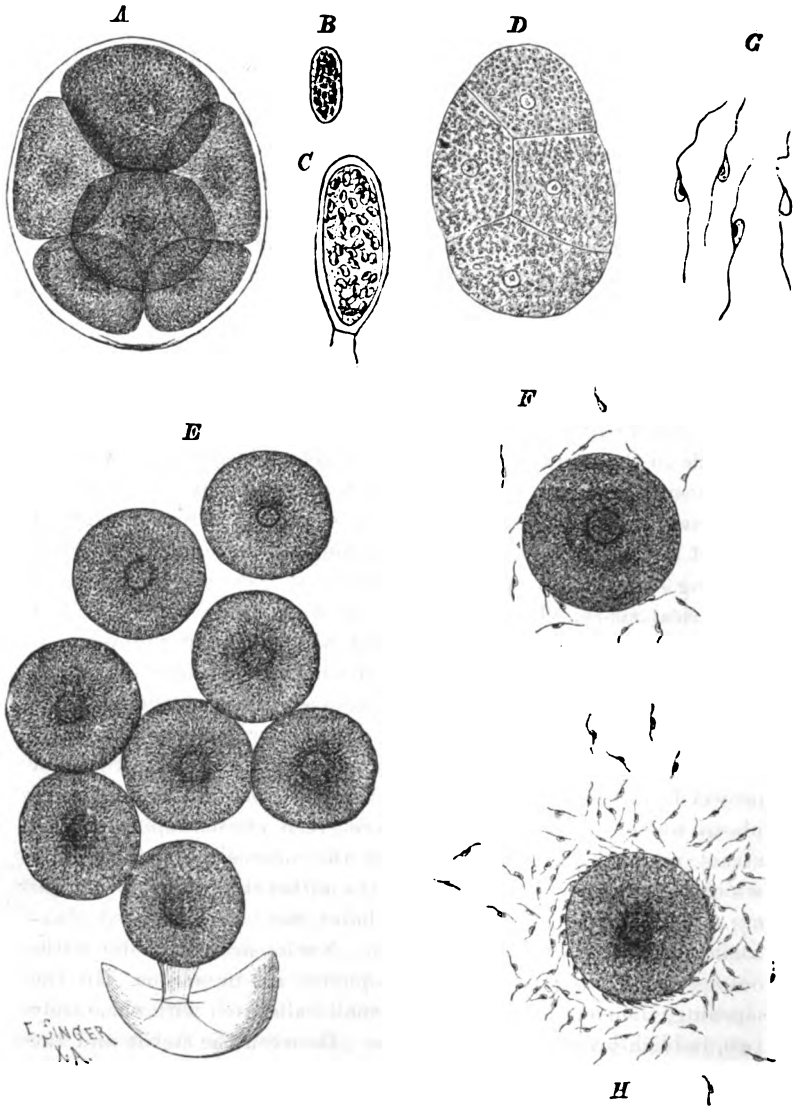


FIG. 87*.—A to F. *Fucus platycarpus*. *A*, evacuated contents of an oogonium, surrounded by the inner cell-wall; *B*, evacuated contents of an antheridium, surrounded by the inner cell-wall; *C*, an antheridium, fixed with alcohol and stained with logwood; *D*, cross-section through the similarly fixed and stained contents of an oogonium; *E*, evacuated oospheres, and a remnant of the case of the oogonium; *F*, oosphere, with adhering spermatozoids.

G and *H*. *Fucus vesiculosus*. *G*, spermatozoids, fixed with iodine; *H*, oosphere, with adhering and swarming spermatozoids. (*C* and *G* × 540; the other figures × 240).

are unicellular, colourless in their periphery, transparent, with a brown fleck in the middle due to the aggregation of the chromatophores. Older stages show from two to eight such flecks, and finally partition-walls are produced simultaneously between these flecks, dividing the contents of the oogonium into eight pretty symmetrically arranged oospheres. After complete division, the brown colour is uniformly distributed through the contents of the oospheres. If the section has passed through the point of insertion of an oogonium, it will be seen that it is situated on a short unicellular stalk.—In almost all sections through ripe conceptacles single oogonia will be torn off from their stalks. If such oogonia are observed for a time, an outer layer of the wall is seen to rupture at the apex, and the oospheres protrude, surrounded by an inner layer. This inner layer swells strongly in water, especially at its upper part, and gradually becomes invisible, and the oospheres are distributed in the surrounding water (Fig. 87* *E*). The oospheres round off, are devoid of membrane, and in the centre of each a clearer fleck is recognisable. If ripe antheridia are similarly broken off, after a time the contents are extruded, surrounded by an inner sheath (Fig. 87* *B*). After a time the contents escape in the form of small pear-shaped bodies, but in these cases movement is not usually seen.—Material hardened in alcohol cuts much better, and, if stained with hæmatoxylon, gives beautiful figures, which amplify in not unimportant points the results obtained from fresh material. Thus, nuclei can be detected in the rudiments of oogonia, in number increasing, by repeated bipartitions, from two to eight; then follows the simultaneous formation of dividing walls, each oosphere having a central nucleus (Fig. 87* *D*). The nucleus always corresponds in position with the brown fleck referred to above, so that we assume that each of these latter encloses a nucleus. The position of the nuclei is seen particularly clearly in the antheridia. In the ripening antheridium (Fig. 87* *C*) we can determine that almost the whole body of the spermatozoid consists of nuclear substance, which is surrounded by only a thin layer of protoplasm. The whole of the protoplasm is not thus used up, a little, not staining with logwood, is left between the spermatozoids. As a fixing reagent, and to some extent better than alcohol, can be used 1 per cent. osmic acid, picro-sulphuric acid, boiling water, or bromine vapour. Fixing with bromine vapour and boiling water often gives the best results, and is especially advantageous in that it does not require a subsequent

washing of the preparation. The preparations can afterwards be stained with logwood, saffranin, gentiana-violet, or carmine, for particulars of which see *Cladophora*, *Spirogyra*, and "Cell-division." The stained preparations are then dehydrated and decolorized by 50 per cent. alcohol, which is gradually replaced by absolute, then cleared with oil of cloves or oil of marjoram, and mounted in Canada balsam or dammar.

In the plants left hanging in free air, after some search will be found sexual organs, which have escaped from a conceptacle. They appear as small olive-green drops of mucilage at the mouths of the conceptacles, and in which the oospheres can be seen even with a lens. If such a drop of mucilage be removed with a needle, and placed in a drop of sea-water on a slide in a moist chamber, we shall see numerous oospheres and spermatozoids still enclosed in the inner sheaths of the sexual organs, and which repeat the phenomena given above. Soon, however, the antheridium-cases commence to empty, at one, or, less often, at both ends. The spermatozoids can commence to move before, upon, or not for some time after evacuation. The movement (Fig. 87* *G*) is very active. They may swarm for several hours, but usually for much less. They are pear-shaped, with two unequal cilia, the shorter one attached to the anterior or pointed end of the spermatozoid, and directed forwards, while the longer one is attached laterally, and directed backwards. The reddish-brown fleck or spot is at the point of insertion of this posterior cilium. The spermatozoids can be quickly and advantageously fixed with iodine. Freed oospheres may be seen surrounded by swarming spermatozoids, many of which cling to its bare surface (*F*). They attach themselves obliquely by the pointed end and a portion of the long side, so that the hinder cilium remains free, and for some time can be in motion. If a sufficient number are present, they can give to the oosphere a rolling motion. This phenomenon is noticeable in the processes of fertilization of various sections of the animal kingdom, *e.g.*, in the Echinoderms, the Actinia, and Vermes; but is known amongst plants nowhere else than in the Fucaceæ. This movement lasts from ten to twenty minutes, then the oosphere comes to a rest; a spermatozoid has penetrated and fertilized it. A cell-wall is then formed around the fertilized oosphere, or **oospore**. If fertilized oospores are kept in a watch-glass with sea-water, at the second, or, latest, the third day, the first divisions of the oospore can be made out. Unfertilized oospheres usually fall

quickly to the bottom, without covering wall or division. As antheridia and oogonia are found in the same conceptacle, fertilization of the oosphere by spermatozoids of like origin can often result; but, nevertheless, fertilization by spermatozoids from remote conceptacles is by no means excluded, and may be facilitated by the fact that the spermatozoids are usually evacuated before the oospheres of the same conceptacle, and often when the latter are evacuated have already swarmed out.

For the study of the sexual processes, *Fucus vesiculosus* is still more favourable than *F. platycarpus*, and is likewise very common. The structure of the sexual organs is much as in the latter plant, but one sex only, either antheridia or oogonia, is found in each conceptacle. Plants hung up empty their sexual organs after a few hours. The drops of mucilage which contain the spermatozoids are recognisable even to the naked eye from their orange-red colour, while those which contain the oogonia are coloured olive-green. If a little of the orange-red mucilage is placed in a drop of sea-water, this will usually be seen almost immediately to be filled with actively moving spermatozoa. Thoroughly healthy spermatozoa are tolerably sensible to light, and nearly always avoid it (are negatively heliotropic, or apheliotropic), so that even with slight illumination they usually collect at the room side, rarely at the window side, of the drop. With intense light their movement is fairly rectilinear, in the direction of the incident rays. Single spermatozoa from time to time stop suddenly, and move for a little while in the opposite direction, but ultimately they all get to the shaded side of the drop. With very weak illumination a definite direction to the movement is hardly recognisable, and the same is the case with unhealthy spermatozoids. As the oospheres are specifically heavier than sea-water, the apheliotropism of the spermatozoa takes them away from the surface of the water, and therefore in the direction in which the oospheres are likely to be.—The spermatozoa can be best fixed with iodine and picric acid, and show the same structure as those already studied (*G*).—In order to see the processes of fertilization, we transfer the olive-green mucilage from the female conceptacle to drops of sea-water on a number of object-slides. We examine these in order to determine the time at which evacuated oospheres are present. Such ought always to be found within the first hour, but oospheres which have been evacuated for several hours are still capable of fertilization; so that we place our preparations in a

dark chamber, and can use them for observation one after the other.—If we follow under the microscope the method of evacuation of the oospheres, we shall see, in contradistinction to *F. platycarpus*, that the case of the oogonium remains visible up to the setting free of the oospheres, that the inner layer is specially clearly marked, and that during the evacuation of the oospheres the outer layer is turned over.—If we bring a little of the orange-red mucilage into a preparation with freed oospheres, these are quickly surrounded by spermatozoids. By turning the preparation in such way that the spermatozoids, avoiding the light, come into contact with the oosphere, we can determine that even those spermatozoids which are removed from the oosphere by a distance equal to the diameter of these latter, suddenly turn from their path in order to rush towards the oosphere. This attraction extends over approximately a double diameter of an oosphere. As has been recently determined by Pfeffer, this attraction depends upon a body given off from the oosphere, which acts as a chemical stimulus in determining the direction of movement of the spermatozoids. The spermatozoids cling to the oosphere, which is soon completely covered with them. The spermatozoids lie obliquely on the surface of the oosphere with the beak and a portion of that side which is devoid of cilia, while the hinder, laterally inserted cilium continues for a time its vibratory movement and sets the oosphere in rapid rotation. The rotation takes place in the direction in which the beaks of the majority of the spermatozoids are directed; should the direction of rotation change, it is due to the clinging of new spermatozoids, which alter the orientation of the majority. The movement is no doubt the resultant of the component movements of the spermatozoids; but if the movement tends to maintain any uniform direction, the spermatozoids arranged in any other direction tend gradually to alter their position and to assume one corresponding with the movement. In addition to the attached spermatozoids, the oosphere appears to be surrounded by a swarm of free spermatozoids, which move inside its sphere of action (Fig. 87* *H*). In from ten to twenty minutes the rotation ceases, and the clinging spermatozoids have left the oosphere. In the meantime **fertilization** has been effected, a spermatozoid being no doubt absorbed, although, owing to the opacity of the oosphere, this cannot be directly observed. The **fertilized egg**, or **oospore**, has likewise developed an exceedingly delicate membrane.—If a quantity of oospheres are mingled, as

above, with spermatozoids, either on a hollow object-slide or watch-glass, and after a few minutes fixed and stained with iodine solution, we may be able to see the result of fertilization. In most of the oospores two nuclei can be seen: a larger one, with large nucleolus, the **oo-nucleus**; and a usually somewhat smaller one, with smaller nucleolus, representing the **spermo-nucleus**, or nucleus of the absorbed spermatozoid. The penetration of the spermo-nucleus towards the centre of the oospore must take place very rapidly, for we find it commonly already in the neighbourhood of the oo-nucleus. In some of the oospores the two nuclei have already been combined into a single one, in which process lies the essence of **fertilization**. The **embryo-nucleus**, the result of the copulation of the oo-nucleus and spermo-nucleus, shows at first two, but later on only one, nucleolus.

The sexual organs of the Characeæ, or Stoneworts, are attached to the leaves. *Chara fragilis* fructifies freely at the beginning of summer. The **antheridia** are recognisable even with the naked eye as red globules, with a diameter of about $\frac{1}{60}$ of an inch. They stand singly on the inner side of the leaves, in their middle line (Fig. 87** *A*, *a*), and occupy the position of a leaflet. The female organ, the **oogonium**, or **nucule**, is found close above the antheridium, and arises from the lowest node of the leaf which is developed into an antheridium (Fig. 87** *A ob*). The antheridia have a complicated structure. In order to make ourselves familiar with it, let us take a ripe antheridium under external examination with a magnification of about 100 diameters. It shows then apparently a red centre, surrounded by a colourless sheath. This colourless sheath is segmented by beautifully arranged partition-walls. Now search for an antheridium which is as ripe as possible, and which will be found somewhere upon a leaf the uppermost antheridium of which (which first dehisces) has already fallen to pieces; separate it off with the needles, and crush it carefully under a cover-glass. If the antheridium is thoroughly ripe, its wall falls into regular pieces, the **shields**. From the interior come numerous long, delicate filaments, and between these some cylindrical orange-coloured cells. These last are set into the centres each of one of the shields, like handles, and are known as the **manubria**, and their coloration arises from elongated chromatophores. More careful examination further shows us that each such unicellular manubrium bears at its narrower end a colourless rounded cell, the **capitulum**, from which arise a number of smaller colourless cells,

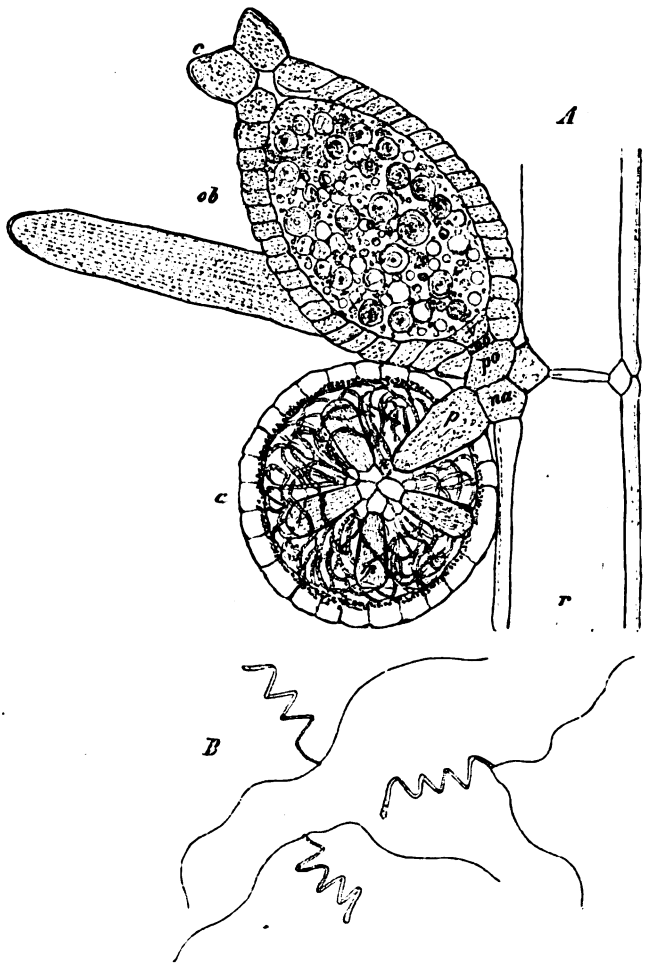


FIG. 87**.—*Chara fragilis*. *A*, median longitudinal section through a leaf, *r*, and the sexual organs arising from it; *a*, antheridium, of which *na* is the basal node, *p* the stalk- and *m* the manubria; *ob*, an oogonium or nucule, of which *po* is the stalk-cell, *no* the nodal cell, *v* the "Wendungszolle," and *c* the crown (× 90). *B*, spermatozoids (× 540).

the **secondary capitula**. From these come the numerous slender, colourless filaments. Even with a power of 200 diameters we can see that each of these threads consists of a large number of flattened cells, forming a linear series. If the antheridium is ripe, we can see in each of these cells a coiled thread, the **spermatozoid**. We now place our preparation in a moist chamber, and endeavour in

another fashion to obtain information as to the structure of the wall of the antheridium. When the antheridium is crushed a clear insight into the structure of this wall is hardly to be obtained; for the natural dehiscence of the antheridium upon the object-slide we should wait in vain; here and there, however, where antheridia have opened a short time before, the segments of the wall can usually still be found. They adhere to the leaf, held together by the disorganised remnants of the threads. We separate one out with the needles, and can now easily study their form and structure. They are triangular, or trapeziform quadrangular. These segments, or shields, are flat, and are traversed by partition walls, which, directed towards a common central point, do not however reach it. Each shield is therefore intercellular, chambered, however, round its margin by the projecting ridges. The ridges answer to indentations of the margin. The shields contain red globular chromatophores, which, separated by the ridges into striæ, come together only in the middle of the cell. They lie close to the inner wall of the shield, whence it came that in examining the entire antheridium a dark red centre appeared to be surrounded by a clear sheath.—If we examine now a young but fully formed antheridium, we can determine that the shields fit together by the indentations of their margins, and that eight shields, to wit, four above and four below, are combined in the wall. The four upper have the form of triangles, the four lower that of trapezes, because an angle of each of these latter is cut off, leaving a short side by which it joins on to the stalk of the antheridium.—A complete insight into the structure of the antheridium can however only be obtained by means of sections. To obtain these is not so difficult as might at first sight appear. A leaf covered with sexual organs is placed between the fingers, the sexual organs turned inwards, and it is then halved longitudinally with a sharp razor. As a rule this suffices, but we can endeavour further to divide one of the halves, so as to obtain a median longitudinal section. If this has been obtained we obtain the structure as shown in the annexed figure (87**). The insertion of the antheridium (*a*) in the leaf is clear. The stalk of the antheridium (*p*), with its wall lined with orange-red chromatophores, just as with the manubria, projects right into the interior of the antheridium. From the centres of the shields spring the manubria (*m*); the capitula seated thereon impinge upon one another and upon the stalk-cell. From the secondary capitula we

can see proceeding the threads of mother-cells of the spermatozoids.—Now let us again take the preparation with the crushed antheridium, which we had laid on one side. If this had been very ripe, the spermatozoids will now, after the lapse of an hour or two, have commenced to free themselves from the threads. They emerge from their mother-cell by a lateral opening, showing brightly through a swelling substance which has been squeezed out from it. They are corkscrew-like threads (Fig. 87** *B*), very like to the spermatozoids of mosses to be studied hereafter. They describe four complete turns; at their anterior, somewhat tapered end they bear two very long cilia, longer than the entire body of the spermatozoid, and readily recognisable after treatment with iodine. At their hinder end the spermatozoids are somewhat thickened, and enclose glistening granules. The spermatozoids progress by simultaneous vibratory movement and rotation around their axis. The whole preparation swarms with the spermatozoids, since the number produced in a single antheridium has been estimated at about 30,000.—The antheridia tend to open spontaneously in the early morning. The spherical curvature of the shields diminishes notably, so that they separate from one another, and this tendency occasions the springing open of the organ. The spermatozoids are wont to swarm for some hours.

In order to inform ourselves as to the structure of the **nucule**, or **oogonium**, we preferably first study it in those stages of development in which it is still cylindrical and transparent. The antheridium found under it is then fully formed, and the oogonium itself begins to become somewhat brownish. In such an oogonium can be seen an elongated central cell, densely filled with finely granular protoplasm, and at the base is shown a clearer spot. It is borne upon two flat internal cells, of which the upper is distinguished as the "Wendungszelle" (*v*), and the under is a nodal cell (*no*), and upon a short stalk-cell (*po*). This last is seated upon the nodal cell (*na*) which bears the antheridium. The central cell of the oogonium is sheathed by five tubular sacs, which arise from the nodal cell (*no*). These sacs run spirally around the central cell, and end above in the so-called **crown** (*c*). The five cells of this latter are cut off by partition walls from the investing sacs. If the crown consists thus of but five cells, we can at once determine our plant to be *Chara*, while the other genus of Characeæ, *Nitella*, possess a ten-celled crown, consisting of five pairs of cells, the result of a subsequent division of each of the five cells.—In the investing sacs of

the sheath of such young oogonia, **protoplasmic streaming** can be very beautifully seen. The chlorophyll grains have already elongated and taken on a brown tone.—In subsequent stages the oogonium becomes oval, and the central cell, the egg-cell or oosphere, becomes filled with oil-drops and starch-grains (Fig. 87**), becoming thus opaque; the starch-grains show a beautiful concentric lamination. The investing sheath becomes darker, and masses of lime become deposited on its outer surface.—The egg-cells are receptive, *i.e.* are ready for fertilization, at the same time when the antheridia of the same leaf open. The investing sacs close under the crown elongate a little, by which the outer layers of membrane of the sacs are torn at this spot. As the result of this we see the sheath, which previously had been incrusted with lime right up to the crown, now become free from lime just under the crown. Simultaneously with their elongation the investing sacs have also separated from one another, and thus clefts arise which reach right into the interior to the apex of the oosphere. By elongation and lateral separation of the investing sacs, therefore, a short "neck" has arisen under the crown, rendering fertilization of the oosphere possible. If, indeed, we examine in the early morning the oogonia found in the immediate neighbourhood of the last opened antheridia, we shall find in and upon the clefts of the neck numerous spermatozoids clinging to them. They are arrested here by a jelly-like substance.—The fertilized egg, or oospore, becomes surrounded by a strong colourless membrane, and the inner wall of the sheathing sacs bounding upon this commences after some time to thicken and become brown. In spite of the incrustation of lime, these relations can be made recognisable by treating the oogonium with hydrochloric acid.

NOTES TO CHAPTER XXII.

[1] De Bary, *Conjugaten*, p. 8; Strasburger, *Befruchtung und Zelltheilung*, p. 5; *Kny*, Wandtafeln, Text, p. 11.

[2] Schmitz, *Stzber. der niederrh. Gesell.*, 4th Aug., 1879, p. 23.

[3] Compare Thuret, *Ann. des sc. nat. Bot.*, III. Sér., tom XIV., p. 219 and plate 16; Schmitz, *Siphonocladiaceen*, p. 34, and *Chromatophoren*, p. 119, Note; Strasburger, *Zellbildung und Zelltheilung*, 3rd Edit., p. 72.

[4] Compare Areschoug, *Observ. phycol.*, II., *Acta soc. scient. Upsala.*, vol. IX., 1874.

[5] Thuret, *Annales des Sciences Naturales, Botanique*, II. Series, tom. XIX., p. 270; Strasburger, *Zellb. u. Zellth.*, 3rd Edit., pp. 213 and 84.

[6] Schmitz, *Stzber. der niederrh. Gesellschaft*, 4th Aug., 1879, separate reprint, p. 4; Strasburger, *Zellb. u. Zellth.*, 3rd Edit., p. 88.

[7] Compare Pringsheim, *Monatsber. der köngl. Akad. d. Wiss. zu Berlin*, of the year 1855; De Bary, *Ber. der Freib. Naturf.* Gesell., 1856; Strasburger, *Zellb. u. Zellth.*, 3rd Edit., p. 90.

Upon *Fucus*, see Thuret, "Recherches sur la fécondation des Fucacées" (*Ann. d. Sc. nat.* IV ser. T. 2, 1854) and "Etudes phycologiques."

CHAPTER XXIII.

THE REPRODUCTION OF FUNGI.

Material Wanted.

Fresh horse-dung to grow moulds upon.
Piece of diseased potato plant. Fresh.
A piece of bread to grow blue mould upon.

If a piece of damp bread is placed under a glass bell-jar, it is covered, even in a few days, with a thick felt of fungus threads, [**mycelium**], which almost always belongs to *Mucor Mucedo*,[1] one of the **Phycomycetes**. This fungus soon shows itself very luxuriantly upon fresh dung, kept in a closed moist chamber. From the substratum arise erect fruiting branches, **conidiophores**, or **gonidiophores**, an inch or more in length, which turn towards the source of light, and end each one with a globular, yellow or brown head, readily visible with the lens and even with the naked eye. If we lift some of this material carefully from the substratum, and place it in a drop of water, we can determine, by means of sufficiently strong magnification, that the mycelium consists of thick, copiously branched, irregularly septate sacs [**hyphæ**], and that from these arise the straight, unseptate and unbranched fruiting branches, which bear each one of the globular heads, the **sporangium**. If still unripe, this remains unchanged in the water; its contents consist of yellowish-brown protoplasm. In the youngest stages the fruit-stalk is not cut off from the sporangium; later on there arises a partition wall, strongly arching into the interior of the sporangium, so that the stalk ends inside the sporangium with a swelling like a ninepin, the so-called **columella**. The ripe sporangium deliquesces in water, and of its wall only fragments formed of fine needles remain behind, of which it has been determined that they consist of oxalate of lime.[2] The expelled spores (**gonidia**) be at pretty regular distances from one another, and by pressure on the cover-glass we can determine

that they lie embedded in a colourless slime. On the stalk, under the columella, is usually to be seen a small collar, as a relic of the lime-crust which was attached there. In the peripheral protoplasmic layer of stalks which are not too old, we can follow fine, in the main longitudinal, streaming of the protoplasm. The sacs of *Mucor* are multinuclear, the nuclei very small, only distinguishable by suitable staining. In dung-cultures the fungus occasionally forms **zygotes** [**zygospores**], which present themselves as dark points. They can usually be forced into the formation of zygotes [zygospores] in the months of March and April, if the spores are sown in fresh, flattened-out horse-dung. The zygotes are ready in from eight to fourteen days. At other times, in order to obtain the zygotes, it succeeds well if the sowing is made in some drops of concentrated plum-juice, sterilized by long boiling, and then mixed with ten to twenty per cent. of alcohol [not methylated]. The sowing is made on a cover-glass in a damp chamber constructed of a glass ring (see p. 244), and the object-slide placed in the large plaster-of-Paris moist chamber (p. 243). The zygotes [zygospores] arise by conjugation of the ends of mycelial threads swollen into club shape. On the ripe black zygotes, covered with warts, the positions of these two mycelial threads can be seen opposite to one another, as clearer places with circular outline.

If uninjured material is transferred to absolute alcohol, chromic acid, or picric acid, and afterwards stained, the lining plasmic layer of the mycelium, as of the aerial gonidiophores, can be seen to contain numerous small nuclei, scattered at irregular distances, and connected together by plasmic threads. They are also recognisable in the sporangia, and, though with more difficulty, in the spores. In these latter there is usually but one, sometimes two.

Mucor Mucedo is a very suitable object wherewith to be introduced to the methods of fungus culture upon the object slide, and we will therefore amplify here the methods already learned in connection with bacteria. We prepare a suitable culture-fluid, by boiling an infusion of horse-dung in water. The infusion is filtered clear, and then again boiled for a long time in order to sterilize it. The object-slides and glass-utensils needed for use must likewise be sterilized by passing through a gas or spirit-flame, or by being laid for a short time in absolute alcohol and then in ether, which latter will rapidly evaporate after removal. It has also been recommended to preserve the glass-utensils in 10 per cent. hydrochloric acid, to remove them just before needed for use, and wash

them in distilled water, which has been boiling for some hours. Glasses cleaned in this way allow the drop of culture-fluid to be well spread out, a point of no small advantage. These precautions are rendered necessary by the existence in the atmosphere of various spores which might infect the culture. It is necessary now to sow a single gonidium, and this is effected in the following way. A sporangium from a pure culture is transferred with the forceps to a watch-glass filled with boiled water. In this the gonidia will soon become uniformly diffused. A drop of the fluid can then be taken out of the watch-glass by means of a needle which has been disinfected in a flame, and laid in an elongated streak upon an object-slide. This streak is then examined under the microscope. If it contains but one gonidium, it is in a fit state for use for the culture; but if it contains more than one, a part of it must be wiped off with a scrap of rag. A drop of the culture-fluid must then be laid on the gonidium, the slide then laid upon one of the zinc frames represented in Fig. 1, and this covered with a bell-jar, the edges of which are immersed in water.—It is even better to add a few drops of the culture-fluid to the watch-glass of water containing the gonidia, and to leave them there for a few hours. The gonidia swell to a ten-fold size, becoming globular, and are much easier to see and count in the streak upon the object-slide. The swollen gonidium will show a large central vacuole (Fig. 87c, *B*). Several germinal tubes will quickly be developed from the gonidium, will grow rapidly, and, in the course of a day, as can be readily seen by repeated examination under the microscope, will produce a highly-branched mycelium (Fig. 87c, *C*). The successive systems of branches progressively diminish in thickness. The entire mycelium has no partition walls, and is filled with dense, granular protoplasmic contents, in which are numerous vacuoles. When the mycelium has attained a definite size, further branching ceases; the protoplasm becomes more granular and darker, and begins to collect towards the middle of the mycelium. Here the gonidiophore is erected out of the fluid as a thicker branch; and when this has attained a certain size, the

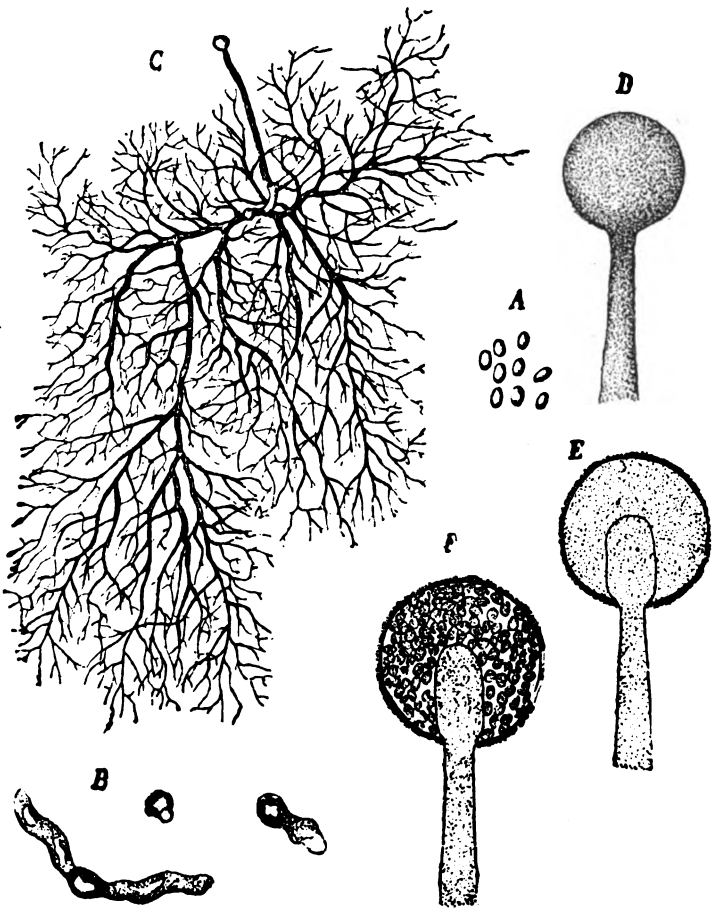


Fig. 87c.—*Mucor Mucedo*. *A*, spores; *B*, germination of spores; *C*, unicellular branched thallus (mycelium), proceeding from the spore, and commencing to produce erect sporangiferous hyphæ (gonidiophores), which are developed centrifugally; *D*, swollen end of an erect hypha, still continuous throughout; *E*, separation of the sporangium by a partition, so as to form the columella; *F*, formation of spores, separated by an interstitial substance; the wall of the sporangium bristles with crystalline points of oxalate of lime. (From Van Tieghem's *Traité de Botanique*, after Brefeld.)

end swells into a head (Fig. 87c, *C*), the bulk of the protoplasm of the mycelium moves towards this rudimentary sporangium (Fig. 87c, *D*), and is replaced by cell-sap. The sporangium is cut off by a partition wall, which bulges into it (*E*), and its contents separate into individual portions, which constitute the gonidia (*F*).

When the sporangium is ripe the gonidiophore rapidly elongates greatly. In the mycelium, partition walls have already been formed. This stage of the development is attained at the outside in three days.—While we should by no means neglect to get this insight into the rapid development of this fungus, it is necessary to state that should we wish to carefully study the various stages as above, it will be necessary to start several cultures, since for observation a cover-glass must be laid on, and the preparation will thus be spoiled for further development. With a sufficiently high power, protoplasmic streaming will be readily recognisable, and especially along the wall of the gonidiophore. Permanent preparations of isolated spore-cultures, at any time prior to the elongation of the gonidiophore, can be made by fixing the object by carefully flooding the object-slide with one of the fixing fluids, and subsequently likewise staining the object upon the slide. In the centre of such a preparation the original gonidium can usually be still recognised as a slight swelling (*see* Fig. 87c, *C*).

On the object-slide we obtain only gonidiophores, ultimately several on each individual; in order to see the sexual organs and zygospores, we must make a culture *en masse*. They are most likely to come upon horse-dung cultures, but rarely in quantity; so that we often look for them in vain. When present, the zygospores show on the dung as black dots. If such a dot be carefully removed on to an object-slide, we can, if it is actually a *Mucor* zygospore, recognise it as a black ball covered with wart-like projections. It is very readily torn, but if it should happen to be complete, we can recognise the two ends of darkly-coloured mycelial threads attached to it (Fig. 87D, *C*). If the mycelial thread is torn off, or has already separated from the zygospore, the places of attachment can be seen as clear circular spots (Fig. 87D, *D*). These can be seen with special ease when the zygospore is crushed. The contents of the zygospore consist, as is then seen, of finely granular protoplasm and oil. Besides the ripe zygospores, we may also find younger, lighter-coloured, or even colourless ones, which do not yet possess the warty prominences, and even we may have

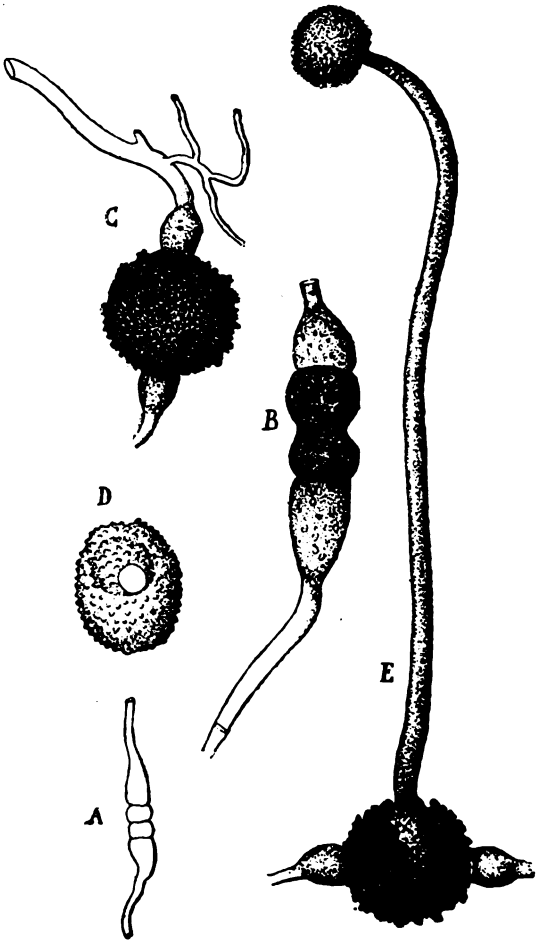


FIG. 87D.—*A*, contact of two branches, and separation of two cells, which will form the zygospore; *B*, fusion of the cells, enlargement of the zygospore and of the branches which bear it; *C*, ripe zygospore, enclosed in the blackened membrane of the conjugated cells; *D*, zygospore isolated from this outer membrane, and upon which we see one of the rings of attachment; *E*, zygospore germinating in moist air into a sporangiferous hypha. (From Van Tieghem's *Traité de Botanique*, after Brefeld.)

portions of mycelium in which the formation of zygospores has just commenced. We shall then see two mycelial threads, rich in contents, the ends of which have swollen globularly, which have joined together by their apical surfaces (Fig. 87D, *A* and *B*). At a little distance from these apical surfaces each of the swollen

ends has been cut off by a partition wall. In somewhat older stages, the contact surfaces of the two sexual organs are wanting, and the contents of both cells have mingled. This zygospore, resulting then from the copulation or **conjugation** of two similar cells, rounds off and enlarges considerably, and the two attached, club-like, swollen mycelial threads form the **suspensors**.

That these zygospores actually belong to *Mucor Mucedo* can be determined by germinating them. When the conditions for their development are present, the zygospores are produced in great number. A large amount of material for investigation can be obtained by cleansing the dung in question with water. The ripe zygospores sink. They are carefully washed, and laid upon object-slides under a bell-jar with its edges immersed in water. Germination begins in about six weeks, when each zygospore emits usually one thick germ tube, which is a gonidiophore, and is crowned by the usual sporangium of *Mucor* (Fig. 87D, *E*). For the emission of the germ tube the black outer wall of the spore, the **exosporium** is only torn so far as is necessary; the development of the gonidia proceeds relatively slowly, so that it is completed about the third day after the commencement of germination.

Mucor can be grown also upon the surface of a saccharine fluid, and forms submerged and aerial hyphæ as upon bread or dung. But if spores are grown completely submerged in such a fluid, as, for example, in a thin layer between a cover-glass and a slide, the hyphæ produced break up by constriction into necklace-like strings, the units of which separate and bud much after the fashion of *Saccharomyces* (p. 215), and like it induce active fermentation.

In studying dung-cultures of *Mucor Mucedo*, it is well to note that it is commonly accompanied by two other Mucorineæ, *Chætocladium Jonesii* and *Piptocephalis Freseniana*, which grow parasitically upon it. The mycelial threads of *Chætocladium* unite with the mycelial threads and gonidiophores of *Mucor* by means of the resorption, at the place of union, of the separating walls. Numerous further prominences arise, and unite with the *Mucor* hyphæ as suctorial apparatus, or haustoria. The mycelial threads of *Piptocephalis*, on the other hand, cling to the *Mucor* threads by swollen ends, from which numerous delicate processes penetrate into its interior. If note is not made of this the fructifications of these

two parasitic fungi may be taken as belonging to the *Mucor* itself.

In the cultivation of fungus spores in moist chambers it may be remarked that for rapid cultures the pasteboard chambers answer admirably; but that for cultures which last more than a few days they cannot be used, as they themselves form a nidus for the spores of various moulds. Glass chambers must then be used. The infusion of horse-dung recommended for *Mucor* does not "keep" for long, and therefore can be recommended only for cultures of rapidly developing moulds. If the development requires a longer period, it is sometimes feasible every other day to remove the drop carefully with a pipette, and replace it with a new supply. The infusion keeps the longest when the dung is stirred in water, boiled, and filtered, and the filtrate kept for at least twenty-four hours in a vapour-bath. In many cases a cold extract of dried fruits, such as raisins, pears, or plums, is very serviceable. Such an extract is filtered till clear, and then evaporated to the thickness of syrup. It can be kept for years unchanged, and when wanted for culture purposes can be mixed in suitable proportions with well-boiled water. If the fluid has an acid reaction it must under some circumstances be neutralised with ammonia, as many fungi cannot endure the acids obtained from fruits.

The cause of the potato-disease is likewise a Phycomycete, the *Phytophthora infestans*, de Bary,[3] germinating hyphæ of which penetrate through the membranes of the epidermal cells of the leaf into its intercellular spaces, and spreading about in these destroy the tissue of the host,* forming brown spots of constantly increasing diameter. In order to obtain the fungus fructifying in large quantity, we place a piece of a diseased potato-plant in an atmosphere saturated with moisture under a bell-jar, and let it lie there for about two days. The diseased leaves are now covered over on both sides, but especially on the under, with white "mould," formed by the filamentous fruiting branches [**gonidiophores**] of the *Phytophthora*. These tufts of mould are especially developed at the edges of the brown spots. On surface sections of the parts covered with mould we see the gonidiophores† pro-

* I have thus rendered the Author's term "Nährpflanze," as the word "host" is fully incorporated into English scientific phraseology as signifying the living organism upon which another organism, animal or vegetal, lives, and more or less completely preys. [Ed.]

† In the Author's corrections for the English edition, he has throughout, following the terminology of de Bary, erased the term "conidia," and inserted

jecting through the widely opened **stomata.** We can demonstrate this, though less completely, in fragments of leaves, which we place in their entire thickness under the microscope. The gonidiophores appear as delicate, unseptate threads, branched above, and filled with finely granular protoplasm (Fig. 88, *A*). The branching is **monopodial** or racemose; the number of branches usually but two or three. These branches are irregularly swollen at intervals. In dry air the gonidiophores, collapsing, are twisted upon their axis. Here and there we see at the end of a branch a **gonidium** in course of development; the ripe lemon-shaped gonidia, however, have fallen off in laying the preparation in water. In order to find the gonidia on the gonidiophore, we must examine the preparation dry. The preparation is, however, to be covered with a cover-glass, and a trace of water placed under it from the edge, because otherwise the gonidiophores, as already indicated, rapidly drying, shrivel up. In plants collected from the open air the gonidiophores are found only on the under side of the leaves, and do not grow so tall as in the moist chamber; are much less noticeable, therefore, with the naked eye. Delicate cross-sections through diseased leaves, made by means of elder-pith, and always at the margins of the spots, permit us to clearly follow the exit of the gonidiophores from the stomata. Often several such hyphæ come side by side out of the same stoma; or, more commonly, the hypha branches at its exit, and gives correspondingly more gonidiophores. From these places we can, though with great difficulty, follow the hyphæ inwards, into the tissue of the leaf, and determine that they pass into the **intercellular spaces.** As a distinction from the most nearly-allied species of *Peronospora*, *Phytophthora* forms but sparingly, and then only short, **suctorial organs** (**haustoria**), penetrating into the cells of the host, so that usually they may be looked for in vain. The delicate mycelial threads, on the other hand, cling closely to the cells of the host. Such cells show first a browning of their chlorophyll-grains; these fuse finally together, and with the other constituents of the cell, into a dark-brown, coagulated mass; at the same time the whole cell collapses. The gonidia are lemon-shaped (Fig. 88, *B*), with short stalks, somewhat tapering **apex,** and finely granular contents. The membrane of the gonidium is very delicate, a little swollen at the apex. They are, as we

"gonidia." I have retained this alteration. In most works, however, the student will find "conidia," with its derivatives, applied to Fungi, "gonidia," and its derivatives, applied to Algæ—a separation not without advantage. [ED.]

have already seen, situated at the ends of the branches of the gonidiophore; if they have attained their full dimensions, the apex of the branch under the point of origin of the gonidium further grows unilaterally, presses the gonidium over to one side, so that this comes to lie in a position at right angles with the branch. At the apex of the branch soon arises the rudiment of a new gonidium (compare Fig. 89, *A*). We sow the gonidia in a drop of water upon a cover-glass, and take care by stirring the drop that the greater part of the gonidia are immersed. The cover-glass is laid upon a small moist-chamber, and the drop suspended from it. The culture must not be carried on in too intense light. After the lapse of about an hour, perhaps later, the formation of **swarm-spores** from the contents of the gonidia begins. The gonidia are converted, therefore, into **sporangia**; they can, however, germinate directly, when we see some of those lying at the surface or at the edge of the drop put out a hyphal sac from the anterior papilla. In those that are immersed and form swarm-spores, the contents divide into an indefinite number of cells (*C*), in each of which we can see a small central vacuole. The apex of the gonidium swells out into a papilla, is finally dissolved, and the separated masses of its contents are successively pressed out through the small round aperture. They hasten away as swarm-spores. If we fix these

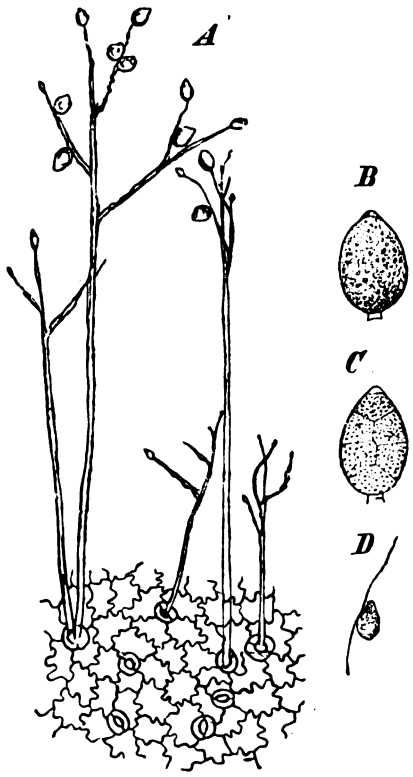


FIG. 89.—*A*, surface-view of the epidermis of the leaf of *Solanum tuberosum* [the potato], with the gonidiophores of *Phytophthora infestans* projecting out of the stomata (× 90); *B*, a ripe gonidium; *C*, another, with divided contents. *D*, a swarm-spore (*B–D* × 540).

swarm-spores with iodine solution, we can determine the presence upon them of two cilia. These are inserted laterally in the proximity of the now peripheral vacuole (*D*). The movement of the swarm-spores lasts up to half an hour. They then come to rest, surround themselves with a cellulose membrane, and germinate soon into a hyphal sac. It is this sac, developed directly from the gonidium, or from a swarm-spore, which penetrates through the epidermis into the stem and leaves of the potato-plant, and can, as may be proved, in this way infect a completely healthy plant. The rapid multiplication of the parasite is provided for by the formation of gonidia.

Sexual organs have not yet been found upon *Phytophthora infestans*, although known for the nearly allied Peronosporeæ. In these, mycelial branches in the interior of the host swell, usually at their end, globularly, and form the **oogonia** by cutting off these swellings by partition walls. By each oogonium is found a mycelial branch, with its end cut off as an **antheridium**. The greater part of the protoplasm present in the oogonium forms a central globular egg-cell or **oosphere**. The antheridium puts out a fertilizing sac to the egg-cell, and this surrounds itself afterwards with a firm membrane.*

Upon the most variable objects in damp positions, even if only traces of nourishment can be obtained from them, soon is wont to be found the blue-green mould, *Penicillium crustaceum*, Fries.[4] It is the most widely distributed of all moulds; we meet with it everywhere. We shall not, therefore, need to seek long for material for examination. It will be, however, most convenient to moisten a piece of bread, and place it under a bell-jar. Not improbably Mucorineæ will first show themselves on the bread; but soon the, at first, more slowly developed *Penicillium* will have supplanted it, and after about eight days covers the substratum with a dense, blue-green covering. The blue-green coloration arises from the spores of *Penicillium*, which, however, only show this coloration when in great quantity. We now lift a little material from the substratum, and examine it in water. The **mycelium** consists of branched hyphæ, which are divided by partition walls. The contents directly visible are finely granular protoplasm and small vacuoles. Individual threads, not distinguishable from other mycelial threads, have developed into fruiting

* In our customary terminology, by fertilizing, the egg-cell (oosphere) becomes an egg (oospore). [ED.]

branches [**gonidiophores**]. At their apex they bear a whorl of short branches, which branches (Fig. 90, *s'*) on their part either bear directly whorls of **basidia**, or previously each one again bears a whorl of shorter lateral branches, and then these bear the whorls of basidia. This branching gives to the fruiting branch the appearance of a brush. Commonly other lateral branches, which arise just under a partition wall of the primary fruiting branch (as in the right hand of the figure), come up laterally to this terminal brush, and form secondary fruiting branches. The basidia, as sufficiently strong magnification shows, are cylindrical, prolonged at their end to a finer projection, the **sterigma** [pl. sterigmata]. This sterigma swells to a globular point, and forms a quickly-growing spore. Under the first spore soon shows a second swelling, which becomes a spore, and so on, so that chains of spores arise, the terminal spore being the oldest. The uppermost spores of the chain are thrown off, while new ones press outwards from below. Tufts of *Penicillium*, fixed with alcohol, stain very well with very dilute hæmatoxylin, by which it can be determined that in the cells of the mycelium and the fruiting branch numerous nuclei are present.[5] The nuclei are very small, so that they require strong magnification. They are elongated in the longitudinal direction of the cell, and joined by fine plasma-strings. In long cells very many can be counted; in the shorter branches of the whorls on the fruiting branches, only one or two; in the basidia, pro-

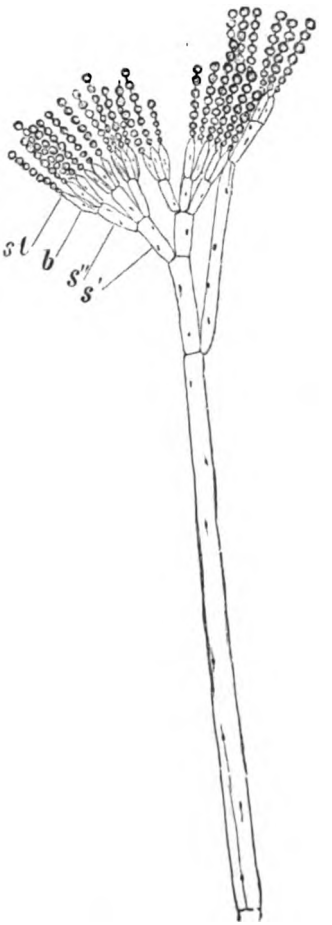


FIG. 90.—*Penicillium crustaceum*, fruiting branches with verticils of branches (*s'* and *s''*), basidia (*b*), sterigmata (*st*), and spores; nuclei visible. From an alcohol-hæmatoxylin preparation (× 540).

bably only one at the upper end. The basidia, however, are usually filled so thickly with contents at the apex that the identification of the nucleus in them is impossible. In the spores also, with the strongest magnification, a nucleus can be distinguished with certainty for each.[a]

To complete what we have already said, it may be added that besides the above-described fruiting branches, it is possible to rear upon Penicillium a second kind of fruiting body.[6] These arise in suitably managed culture *en masse*, have the size of small pin-heads, and a yellowish colour. In their interior, after longer period of rest, **asci** are formed, each of which produces 8 spores [**ascospores**]. Therefore *Penicillium* must be set down as an Ascomycete, one representing the section of **cleistocarpous Ascomycetes**, with closed fruit-body. Out of the spores developed in the asci the brush-like gonidiophores have been again developed upon the object-slide.

NOTES TO CHAPTER XXIII.

[1] Brefeld, *Schimmelpilze*, Heft I., p. 10; the other literature is given here.

[2] Brefeld, *l.c.*, p. 18.

[3] Compare de Bary., *Ann. des Sc. nat. Botanique*, IV. Series, tom. XX., p. 32, and *Beiträge zur Morph. und Phys. der Pilze*, Heft II., p. 35.

[4] Brefeld, *l.c.*, Heft II.

[5] Strasburger, *Zellb. u. Zellth*, 3rd edit., p. 221.

[6] Brefeld, *l.c.*, p. 39.

Compare Bainier, *Annales des Sciences naturelles, Botanique*, VI. Series, tom. XI., p. 315, for further particulars on the culture of the Mucorineæ.

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[a] In the examination of fresh objects in water, it often happens that air clings very tightly amongst the hyphæ, and hinders observation. Attempts to remove the air have usually disadvantageous effects upon the preparation itself. In order to render the gonidiophores of these and other moulds free of air, as far as they will admit of it, and yet having their natural distribution, the following method may be used: A scrap of the material is laid carefully upon the surface of a drop of glycerine, a drop of alcohol is placed upon it, and the cover-glass is laid on.

CHAPTER XXIV.

THE REPRODUCTION OF THE HIGHER FUNGI AND LICHENS.

MATERIAL WANTED.

Leaves of Barberry with cluster-cups. Fresh (gathered in May or June), dry, or in alcohol.

Plants of grass (wheat or oat, etc.) affected with rust. Fresh (gathered mid-June to August), dry, or in alcohol.

Russula sp. Fresh, or in alcohol. Failing this, the common Mushroom (*Agaricus campestris*). Fresh, or in alcohol.

The Morell (*Morchella esculenta*). Fresh, dry, or in alcohol.

Anaptychia ciliaris, in fructification. Fresh, dried, or in alcohol.

IN the months of May and June are found not infrequently upon the under side of the leaves of the Barberry (*Berberis vulgaris*) orange-coloured warts, which, to the naked eye, appear finely pitted. Examination with a lens shows them as cushion-like yellow swellings, upon which are placed small orange-red cuplets. The corresponding positions on the upper side of the leaf present themselves as reddish spots edged with yellow. Examined with a lens, usually numerous brown points, surrounded with orange-red, show out in the inner parts of them. Individual similar points are often to be found on the edges of the cushion on the under side of the leaf. The cuplets on the cushion of the under side of the leaf are the **æcidium-fruits** of *Æcidium Berberidis*, the "cluster-cup" of the Barberry ; the corresponding points on the spots on the upper side of the leaf, and also upon the edges of the cushion on the under side of the leaf, are the **spermogones** appertaining to them. Together they form the first generation of the common fungus, rust of wheat, etc. (*Puccinia graminis*), belonging to the **Æcidiomycetes** or **Uredineæ**, of which the second generation is gone through upon our corn and other grasses, giving rise to the appearance of the disease called "rust."[1] By means of elder-pith we prepare delicate cross-sections through an infected leaf, and examine them with weak, and afterwards with strong, magnifica-

tion. We assume that fresh material stands at our disposal; the investigation can, however, be carried on satisfactorily upon dried and soaked, and well upon alcohol material. The sections prepared from the fresh leaf are especially clear if we run in a little potash solution. In the uninfected parts the barberry leaf shows, proceeding from above downwards: an **upper epidermis**; a single layer of elongated **palissade-cells**; a loose **spongy parenchyma**, about five cells deep; the **under epidermis**. The cushions of tissue of the infected parts have attained more than double the thickness of the leaf. Upon the palissade layer of the upper side, which is higher, but otherwise appears little changed, impinges a closed tissue, which also shows to be more or less elongated in a direction at right-angles to the surface of the leaf, and from the small development of its **intercellular spaces** is essentially distinguished from the spongy parenchyma of the surrounding parts of the leaf. The epidermis of both surfaces of the leaf has not been affected in the form of its cells. The contents of all these cells are disorganized, and consist partly of colourless oil-drops, partly of greenish-yellow and reddish drops and granular masses, proceeding from the chlorophyll-grains and the cell protoplasm. The entire tissue of the cushion shows its intercellular spaces traversed by delicate, partially-branched fungal **hyphæ**, septate by cross-walls, and containing oil-drops. These extend on both sides to the epidermis. With chlorzinc iodine, as also with iodine and sulphuric acid, blue coloration is not induced, since **fungal-cellulose** rarely shows this reaction. The **æcidium-cups**, as we have them before us in longitudinal section, are sunk above the middle in the tissue of the cushion. We easily determine that the mycelial hyphæ under the cups form a dense, almost pseudo-parenchymatous, layer, from which, perpendicularly outwards, and parallel to one another, rise numerous thicker club-shaped hyphæ, in gap-less union, forming the so-called **hymenium**. These hyphæ, the **basidia**, pass over at their ends into straight rows of **spores**, which at the basidia are colourless and, from mutual pressure, polygonal, but gradually become orange-red and rounded. Higher up the spores separate from one another, and are evacuated from the opened fruit. The observation of the youngest spores upon the basidia convinces us, however, that they are cut off one after another by cross-walls, from the apex of the growing basidia. The unilamellar wall of the fruit (the **peridium**) consists of cells which look very like the spores, but remain polygonal, and do not sepa-

rate laterally from one another. Their fine delicately porous walls are especially strongly thickened on the outer side. The developing peridium pushes back and destroys the surrounding tissue of the cushion, and tears open the epidermis in order to open out to the exterior.—The pear-shaped **spermogones,** especially found upon the upper side of the leaf, are, like the æcidium-fruits, surrounded by a weft of hyphæ, though less strong, from which arise densely-crowded, parallel threads, running towards the middle line of the structure. These threads are very delicate; those found in the upper part of the organ project as delicate bundles towards the exterior [compare Fig. 90* later]. These delicate threads, the **sterigmata,** abstrict at their points exceedingly small, globular cells, the **spermatia,** which, as a slimy mass, are evacuated outwards from the organ. The sterigmata themselves contain orange-red oil-drops, which gives to the entire organ, especially in its outer parts, its special coloration. The spermatia do not germinate; their significance is still unknown. There is a disposition to take them for male sexual products, and to consider that the sexual act leads to the formation of the æcidium fruits.—As already mentioned, the fungus lives as a second generation upon **Gramineæ.** It belongs to the **heteræcious** parasites, which, in contradistinction to the **autœcious,** go through their **alternation of generation** upon different hosts. This can be demonstrated by direct sowing of the æcidium-spores upon seedlings of the cereals.[2]

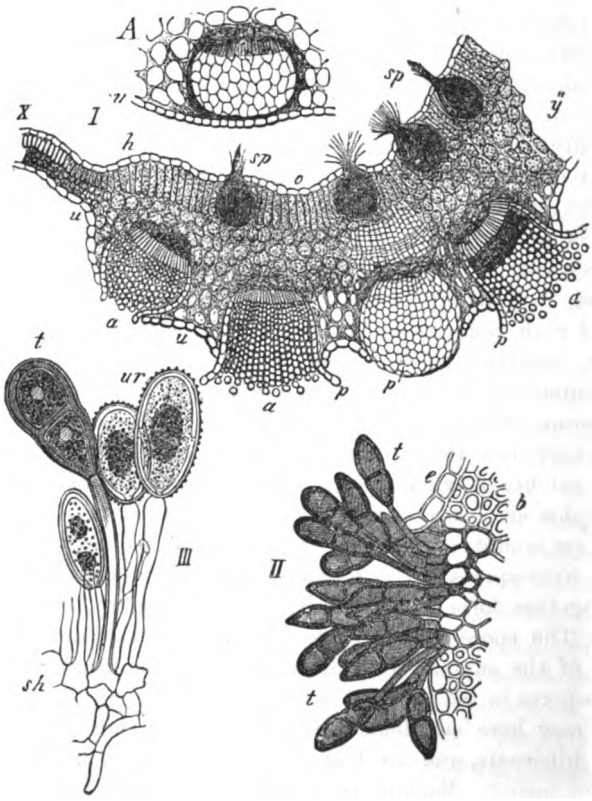
The **uredo** patches of *Puccinia graminis* we encounter not infrequently in the open field, from mid-June to autumn, upon rye, wheat, barley, oats, and especially also on the couch-grass or twitch (*Triticum repens*). They especially take possession of the haulm and the leaf-sheaths of the infected plants. They are easily recognised as narrow, rust-coloured to dark-brown streaks, running parallel with the veins. Upon the leaf-sheaths and haulm (stem) they attain to even two or more inches in length. The epidermis of the host is torn open and raised by the protruding spore-patches. First appear the rust-coloured patches of **uredospores,** with which gradually are associated brown **teleutospores.** They take possession of the patches of uredospores, and at length completely supplant these latter, whereon the patch becomes dark-brown, almost black. Towards the end of summer only teleutospores are to be found.—If fresh material is not obtainable, those preserved in alcohol, and even dry plants, will answer for the investigation. We first prepare a cross-section through the haulm

of an oat which is infected with rusty uredo-patches. We can easily demonstrate upon the cross-section that the fungal hyphæ only traverse definite tissues of the host; it is the chlorophyll-containing, looser strips of tissue, which alternate with sclerenchymatously-thickened strips, in the periphery of the stem, and are covered with an epidermis provided with stomata. Here the cells are densely enveloped in segmented hyphæ, and their contents disorganized. In the places where the section has passed through a patch, we can see numerous short and delicate twigs, directed outwardly, arise from the mycelium, which at their swollen ends abstrict a unicellular spore, the **uredospore.** The surface is ruptured, its edges thrown up laterally. The spores are in different stages of development. Those that are ripe appear an elongated oval, and with sufficiently strong magnification permit us to distinguish two layers in their wall. The outer, dark-brown, is covered with numerous small warts; the inner, less dark, shows several, usually four, regularly distributed pits in the equator. The contents of the spore are granular, in the interior parts a lively orange-red.

Cross-sections through the haulm of oat, bearing the dark-brown patches of **teleutospores,** show the same structure, as far as the hyphæ are concerned, as we have already seen. The teleutospores are mounted upon just the same, but thicker-walled, stalks as the uredospores. The teleutospores are two-celled. The two cells together form an obovate body, somewhat tapering at its two ends. The spore-wall is dark-brown. Plants examined in the course of the summer may have at the same time uredo- and teleutospores in the patch.

We may here supplement this by adding that these teleutospores hibernate, and are first capable of further development in the next spring. Each of the two cells puts out a delicate tube, the so-called **promycelium,** which is segmented into several cells, and from these are put forth short awl-shaped outgrowths, which cut off at their apex a kidney-shaped **sporidium.** These sporidia can only infect *i.e.*, germinate upon the Barberry leaf; if they happen upon a sufficiently young leaf, their germinating tube pierces straight through the outer wall of the epidermal cell directly into the interior of the host. As we therefore see, the way through the stomata, by which the germinating tubes of the æcidio- and uredospores enter, is not the only one by which infection is possible.

[In order further to elucidate the various structures referred to in detailing the life-history of this *Æcidium-Puccinia*, I introduce the adjoining Figure 90*. The description given at the foot of the figure should be compared with that given in the text.]



[Fig. 90*.—*Puccinia graminis* and *Æcidium Berberidis*. I., transverse section of the leaf of *Berberis*, with æcidia (*a*); *p*, the peridium, or wall of the æcidia; *v*, lower, *o*, upper epidermis of the leaf; from *u* to *y''* the leaf has become thickened by the action of the parasite, thus forming the cushion; on the upper surface are spermogonia (*sp*). *A*, a young æcidium which has not yet burst. II., layer of teleutospores (*t*) on the leaf of *Triticum repens*; *e*, its epidermis. III., part of a layer of uredospores on the same plant; *ur*, the uredospores; *t*, a teleutospore. (From Prantl, after Sachs.)]

In order to make ourselves acquainted with the structure of the **hymenium** of the **Hymenomycetes**,[3] we select as best one of the numerous species of Toadstools (*Amanita*), Mushrooms (*Agaricus-Psalliota*), or *Russula*. We select here for examination a *Russula*, because this possesses, moreover, the **cystidia**, which we have at

the same time to make mention of. The cap or **pileus** shows on the underside radially-arranged lamellæ. These bear the **hymenium**. We cut, parallel to the course of the lamellæ, a small piece out of the cap [pileus], and make through this cross-sections perpendicular to the course of the lamellæ; these must be as thin as it is possible to make them. The entire cross-section appears like a comb, on which the sections through the lamellæ form the teeth [see Fig. 91*, *A*, later on]. With a low power we see that the hyphæ pass out of the cap into the lamellæ, run rectilinearly in the middle of these, and gradually give off ramifying branches, which are directed obliquely towards the flanks of the lamellæ, and again branch [see Fig. 91*, *B* and *C*]. Some of these branches swell into club form, and end blind. A large proportion remain slender, and form, outside the club-shaped swollen branches, a dense layer of short, rounded segments, which we will distinguish as the **sub-hymenial layer.** This is limited more or less sharply towards the inner tissue of the lamella —the **trama.** The club-shaped swollen branches of the trama serve to give to the lamella the necessary stiffness. From the sub-hymenial tissue spring the **basidia**, and **paraphyses** (Fig. 90). These have an approximately parallel course, stand out perpendicularly from the flanks of the lamellæ, and form the **hymenium.** The basidia (*b*) are club-shaped. On their flattened ends they form four, symmetrically-placed, thin branches, the **sterigmata** (*c*). These swell gradually at their apices, each into an ellipsoid spore, **basidiospore.** The basidiospores, when they have attained their full dimensions, remain in most cases smooth, or, in many species of *Russula* (cf. Fig. 90), they form short spines on their surface. They are cut off from

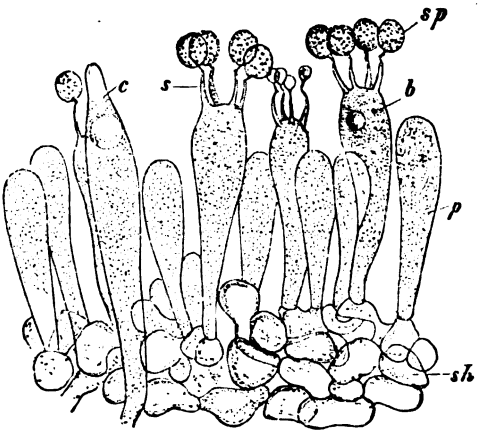
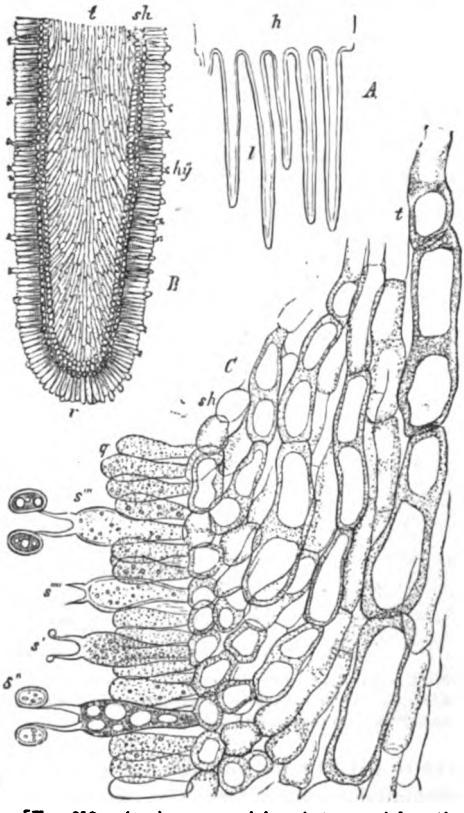


FIG. 91.—*Russula rubra*. Part of the hymenium. *sh*, sub-hymenial layer; *b*, basidia; *s*, sterigmata; *sp*, spores; *p*, paraphyses; *c*, a cystidium (× 540).

the sterigmata by a partition wall, and ultimately fall off. The septation and separation takes place a little below the spore-swelling, in the position where the sterigma shows a slight nick. The fallen spore has therefore a short stalk. Smaller basidia, which remain sterile, form the **paraphyses** (*p*).—So far the Toad-stools and Mushrooms agree with the above-described *Russula*. In *Russula*, however, between basidia and paraphyses occur also individual **cystidia** (*c*), structures of the size of the basidia, which project a little with their pointed ends above the hymenial surface, with their narrowing base penetrate to the sub-hymenial tissue, and show themselves to be direct branches of the median elements of the trama. All the elements in question are bounded at their base by partition-walls; they contain finely granular protoplasm, and not infrequently individual oil-drops.



[FIG. 91*.—*Agaricus campestris*. *A*, tangential section of the pileus (*h*), showing the lamellæ, *l*. *B*, a similar section of a lamella more highly magnified; *hy*, the hymenium; *t*, the central tissue, or trama; *sh*, the sub-hymenial layer. *C*, a portion of the same section still more highly magnified (× 550); *q*, young basidia and paraphyses. At this stage these are practically alike; *s'*, first formation of conidia on a basidium; *s''*, *s'''*, more advanced stages; at *s''''*, the conidia have fallen off. (From Prantl, after Sachs.)]

The accompanying Fig. 91* will further elucidate the structure of the Basidiomycetes. It is taken from the common Mushroom, *Agaricus campestris*. There are no cystids, and each basidium produces only two, instead of four, basidiospores. The

Mushroom offers, moreover, the advantage of being obtainable fresh all the year round.

In order to investigate the structure of the hymenium of a highly-developed form of the Ascomycetes, we will take the Morell, *Morchella esculenta*. Even dried specimens can here, after softening, be employed for the investigation. Fresh are naturally to be preferred. The well-known edible Morell has an irregularly ovate, stalked fructification, which in its interior conceals a simple hollow, and whose upper swollen part has deep folds. The areas or chambers between the ridges or folds are clothed with hymenial tissue, while these are not developed upon the projecting exposed ribs or folds. Suitable sections are very easy to obtain, and they must be taken perpendicularly to the surface of a chamber. The hymenium consists of approximately parallel spore-sacs (**asci**) and **paraphyses** (Fig. 92). The sacs, or asci (*a*) are almost cylindrical, and contain in their upper part eight ellipsoid, unicellular spores, **ascospores**, crowded together. Besides the spores, there is also present in the asci the, in part, strongly refractive **epiplasm.** The paraphyses (*p*) are brownish, septate threads, somewhat swollen above. The uppermost cell is especially long. They do not attain the length of the asci. Asci and paraphyses arise as ends of hyphæ of the densely-interwoven, flatly-extended, sub-hymenial tissue. This arises from the more loosely-constructed inner hyphal texture of the fructification. Addition of potassium-iodide-iodine colours the masses of epiplasma in the asci reddish-brown. This reaction is characteristic of epiplasma, and has been recently pointed out as a reaction for **glycogen.**[4] The characteristic peculiarity of this reaction is shown upon warming. To the preparation lying in water, and stained by the addition of potassium-iodide-iodine, some water is added, yet not so much as to decolorize it; then it is carefully warmed, without attaining the boiling point, and held over white paper, in

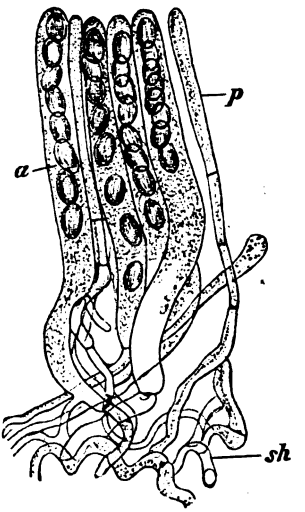


FIG. 92.—Portion of the hymenium of *Morchella esculenta*. *a*, asci; *p*, paraphyses; *sh*, sub-hymenial tissue (× 240).

order to see if the colour has become paler. If this happens, the preparation is then rapidly cooled, and the darker coloration, in large preparations even visible with the naked eye, again appears.[5] With the aid of potassium-iodide-iodine, the base of many asci can be traced pretty deeply into the sub-hymenial tissue. The contents of the spores, paraphyses, sub-hymenial tissue, and of the tissue in the interior of the fructification colours simultaneously yellow or yellowish-brown.

The fungus appertaining to the thallus of Lichens belongs, with few exceptions, to the Ascomycetes. The *Anaptychia ciliaris*, already known to us, fructifies very freely. The **apothecia** are bowl-shaped, with a frame developed from the thallus. This contracts under the apothecium like a stalk. A cross-section through this stalk shows radial structure, with a uniformly thick cortical layer, and following this a homogeneous **gonidial** layer in its entire circuit. The interior of the stalk is occupied by a **medulla**, or "pith," formed of a looser hyphal texture. We further prepare median longitudinal sections through the apothecium. This shows us the frame formed of the tissue of the thallus. The gonidial layer extends to its rim, which grows out in places into ciliary outgrowths. The apothecial stalk has expanded into a bowl-like form, in order to admit the **hymenium**, which arises from the medullary tissue. The hymenium is recognisable by its somewhat brownish colour. It consists of very numerous, long, exceedingly narrow, septate threads, the **paraphyses**; between these, far less numerous, stand the club-shaped sacs, the **asci**. These latter are always of different ages; the ripe ones contain eight brown-walled spores, **ascospores**. These spores are ellipsoid, two-celled, a little constricted at the boundary of the two cells. Paraphyses and asci arise from a like-coloured, felted, horizontally expanded layer of little thickness, which is distinguished as the **sub-hymenial layer**. This originates from the medullary tissue of the stalk, from which it is cut off by its brown colour and the want of air-containing spaces. While, as we have seen, the hyphæ of the thallus itself are not coloured blue even by chlorzinc iodine, those of the hymenial tissue take a dark-blue coloration, even with the addition of a little potassium-iodide-iodine. The walls of the hymenial elements are formed of a special modification of cellulose, which has been distinguished as **starch-cellulose**.—If we examine the thallus of *Anaptychia ciliaris* with the lens, we shall notice in individual spots upon it wart-shaped prominences, standing singly

or in groups. If in such places delicate cross-sections are taken in considerable numbers, we shall probably cut through such a swelling (Fig. 93). It appears then as an ovate structure, sunk in the thallus, and opening outwardly with a pore, and is now known as a **spermogonium.*** It occupies almost the entire depth of the thallus, is laterally surrounded by the gonidial layer, and in the interior shows itself to be constructed of delicate, shortly segmented, approximately radially-arranged threads, singly or in bundles,—the **sterigmata** (compare the Figure). The long axis of the organ is traversed by a cylindrical cavity, which receives the rod-like **spermatia,** which are segmented off from the ends of the sterigmata. Through the upper opening of the spermogone, the spermatia can pass into the exterior. In the Collemaceæ [gelatinous lichens] the function of the spermatia as male sexual organs has been determined[6]; in other lichens their significance is as yet unknown.

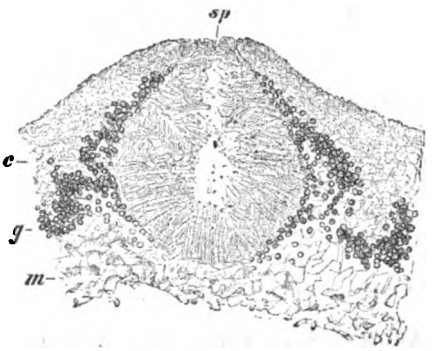


FIG. 93.—Cross-section through the thallus of *Anaptychia ciliaris*.

NOTES TO CHAPTER XXIV.

[1] Compare de Bary, *Monatsber. d. k. Akad. d. Wiss. in Berlin*, for the year 1865, p. 15. Kny, *Bot. Wandtafeln*, text p. 68. Frank, *Die Krankheiten der Pflanzen*, p. 454.

[2] De Bary, as above, for the year 1866, p. 206.

[3] Compare de Bary, *Morph. und Phys. der Pilze*, p. 112; Goebel, *Grundzüge der Pflanzen-morphologie*, p. 143. In both the other literature is quoted.

[4] Leo Errera, *L'épiplasme des Ascomycètes*, 1882. The literature of epiplasm is here given.

[5] Leo Errera, *l.c.*, p. 45.

[6] E. Stahl, *Beiträge zur Entwicklungsgeschichte der Flechten*, Heft. I., 1877.

* As *Anaptychia ciliaris* may not be at the disposal of the student, any one of the following lichens will serve to show spermogones: *Parmelia* (*Physcia*) *parietina*, *Verrucaria nitida*, *Collema melænum*, or *Cladonia rangiferina*. [ED.]

CHAPTER XXV.

THE REPRODUCTION OF MOSSES AND LIVERWORTS.

Material Wanted.

Marchantia polymorpha (Liverwort), with male and female receptacles. Preferably fresh. May be kept in alcohol.

Male "flowers" of a Moss, *e.g.*, *Mnium hornum*, or *Polytrichum*. Fresh, or in alcohol. (*Mnium hornum* is very common in woods and on shady banks.)

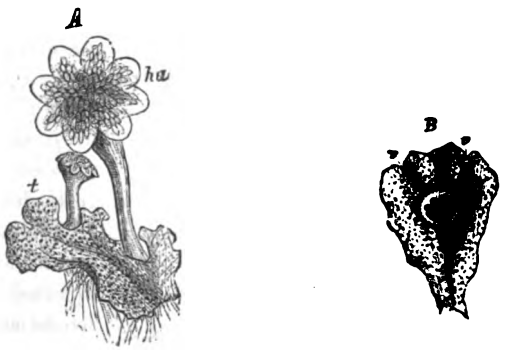
Female "flowers" of the same. Fresh, or in alcohol.

(Both of these gathered in April, May, or June

Spore-capsules of the same. Fresh, or in alcohol.

Amongst Liverworts, *Marchantia polymorpha*, already known to us, rapidly multiplies vegetatively by its **gemmæ**. These are common amongst liverworts in general, and are here met with in especially beautiful form. The gemmæ of *Marchantia* arise upon the dorsal [upper] surface of the thallus in cup-shaped receptacles or **cupules**. [See Fig. 93*, *B*.] The cups have a beautifully toothed rim, and at their bottom the bright green gemmæ are visible. A median longitudinal section through the cupule, parallel to the long axis of the shoot which bears it, shows that the cup is at first slightly narrowed upwards, and then somewhat suddenly expands into the broad rim. The tissue which forms the air-chambers passes into the exterior of the cup, to above the point where its outward broadening begins. The bottom of the cup is occupied by unicellular club-shaped **papillæ**, the membrane of which swells into mucus. Between these papillæ are also found individuals which are two-celled[1]; and some also, the upper cell of which has been further cross-septate. The lower cell remains simple, and forms the **pedicel** (or stalk); the offspring of the upper cell soon divide longitudinally, and the structure becomes constantly more multicellular, enlarges considerably by surface expansion, and becomes several cells thick in the middle. Others will be found which have attained their ultimate biscuit-

like [bi-convex] condition as fully developed **gemmæ**. The unicellular pedicel can easily be broken through. The separation of the gemmæ, and their ejection from the cups, results from the strongly-swelling mucus, which is developed from the unicellular club-like papillæ at the bottom of the cup. Each of the two lateral indentations of the gemma conceals a growing point, protected by short papillæ. The cells of the gemma are rich in chlorophyll, but on both sides large cells, devoid of chlorophyll, occur, which keep near the middle, but otherwise are irregularly scattered. At the edge, individual cells contain oil-bodies. After the dissemination of the gemmæ, the large cells, devoid of chlorophyll, develop in one or two days into hair-roots [**rhizoids**], in all cases on the shaded side of the gemma only, hence becoming the ventral side, while the side exposed to the light forms morphologically the upper or dorsal side.[2]



[Fig. 93*.—*A*, portion of a thallus of *Marchantia polymorpha* (*t*), with the upright male receptacle (*hu*), bearing antheridia. *B*, portion of a thallus with a receptacle containing gemmæ; *v v*, growing points of the two branches of the thallus. (From Prantl, after Sachs.)]

The sexual organs of the Marchantiaceæ are situated upon special receptacles, which we will examine in *Marchantia polymorpha*.[3] **Male** and **female receptacles** are readily distinguishable; the former shield-shaped, with scolloped outline (Fig. 93*, *A*), the latter radiating like bare umbrella ribs. The two sexes are situated upon different plants [the plants are dicœcious]; the receptacles and their stalks are metamorphosed branches of the thallus. We prepare between elder-pith delicate longitudinal sections through the **male receptacle**, and can demonstrate that its upper side has exactly the same structure as the dorsal surface of the thallus,

and that in the same way the under side resembles the ventral surface of the thallus, and bears **rhizoids** and **scales**. On the *upper* side, however, sunk in special cavities, are the **antheridia** (Fig. 94, *A*). On satisfactory sections we can determine that in each cavity is found only one antheridium, besides some short, unicellular paraphyses (*p*); the cavity closes together above the antheridium into a narrow canal. The antheridium is a shortly stalked, oval body, with a unilamellar chlorophyll-containing wall. The **special mother-cells** of the **spermatozoids** [**antherozoids**] have been produced by successive divisions at right angles, and even in the almost ripe antheridium still form rectilineally-arranged cross and longitudinal rows (compare the Figure). Shortly before the ripening of the antheridium, the special mother-cells, rounding off, pass out of union, the wall of the antheridium tears at its apex, and the small, round cells are evacuated. If a drop of water is placed upon a fully-developed receptacle, the water is seen rapidly to spread over its whole surface, and soon becomes milky. If this water is now examined with a high power, we can see in it innumerable evacuated spermatozoidal cells. They remain at rest only a short time, when the cell-membrane swells. Finally it is torn through, and the spermatozoid escapes into the surrounding water. The spermatozoids are comparatively very small, have a thread-like body and two long cilia; to the posterior end clings a bladder, which is lost during the swarming. In order to see them clearly, we run into the preparation a drop of 1 per cent. osmic acid, and as they are fixed beautifully by the reagent, we can now study them conveniently. (Fig. 94, *B*.) We can effect the same purpose by the addition of a trace of potassium-iodide-iodine solution.[a]

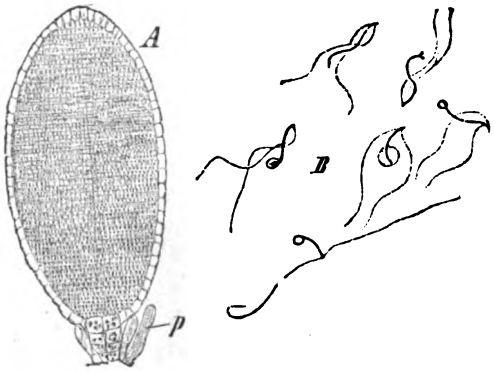


FIG. 94.—*Marchantia polymorpha*. *A*, an almost ripe antheridium in optical cross-section; *p*, paraphyses. B, spermatozoids [antherozoids], fixed with 1 per cent. superosmic acid (*A* × 90, B × 600).

[a] See note on page 286.

The **female receptacle** forms, like the male, a radially-spreading inflorescence, and in general there are nine rays, and *between these* are eight rows of **archegonia** on the *under* side of the receptacle. The distinction from the male receptacles is striking, in that here the sexual organs stand upon the under side; but this phenomenon is connected with an early displacement of the growing point towards the under side of the receptacle. Under the simple microscope we can demonstrate that each row of archegonia, lying between two rays, is enclosed in a common, veil-like covering, fringed at its edges. We prepare, between thumb and forefinger, delicate longitudinal sections through a comparatively young receptacle, and upon some of these sections find, without difficulty, the female sexual organs, the **archegonia.** The oldest lie nearest the edge, the younger progressively nearer the stalk. The first ripening archegonia show alongside the edge of the disk with their neck curved outwards, the succeeding ones hang straight downwards. In an approximately ripe archegonium (Fig. 95, *A*) we can distinguish a short stalk, a ventral portion, the **body,** and a **neck.** The wall of the body, as of the stalk, is unilamellar. The **central-cell** of the body is filled by the egg-cell, or **oosphere,** and the **ventral canal-cell** (*k''*) cut off from it shortly before ripening. In the egg-cell, the nucleus is readily visible. The neck is traversed by the **neck-canal,** which is composed of a series of **neck canal-cells,** the walls between which are dissolved, and the disorganized contents of the four neck canal-cells are thus fused into a connected string. Between the archegonia, numerous small, leaf-like scales of the receptacle can be seen to arise. In many preparations we have in view the unilamellar veil-like covering, fringed at its edges, which protects the entire row of archegonia. Numerous cells of this contain oil-bodies.

It is comparatively easy to see the opening of the archegonium directly under the microscope. We take quickly longitudinal sections through a female inflorescence, which has not yet raised itself, or only a little, upon its stalk, lay it dry under a cover-glass, and examine it under the microscope. When we appear to have found a ripe archegonium, and while still observing, we place a drop of water at the edge of the cover-glass. After the entrance of this, the archegonium opens almost immediately. The cause of the opening lies in the strong swelling of the contents of the neck-canal. The neck-cells separate from one another at the apex of the neck. The contents of the neck canal-cells pass out, then

the contents of the ventral canal-cell follow. The homogeneous portion of these contents is formed of a strongly-swelling slime, which diffuses in the surrounding water; the granular contents remain in the surrounding water, where they are slowly disorganized. Immediately after the ejection of the ventral canal-cell, the egg-cell in the central part of the body rounds off (Fig. 95, *B*). At its anterior margin, *i.e.*, that in apposition to the canal, a clearer spot, the **receptive-spot**, is often, though not always, to be distinguished. Moreover, the penetration of the spermatozoids into the neck-canal can in this plant be easily observed. For this purpose, instead of pure water, we add to the preparation a drop which has previously lain on a male receptacle. The spermatozoids quickly collect in the slime expelled from an archegonium; we see them enter the neck, where they become invisible. A substance is given off from the archegonium, which acts as a chemical stimulus, and determines their direction of movement. Thus they get into the slime given off from the archegonium, in which they slowly move in the direction of the opening of the neck. It is interesting to prove that in an unfertilized archegonium the neck does not close, and under such conditions the archegonium slowly decomposes. If, on the other hand, water, containing spermatozoids is added to the preparation, and the

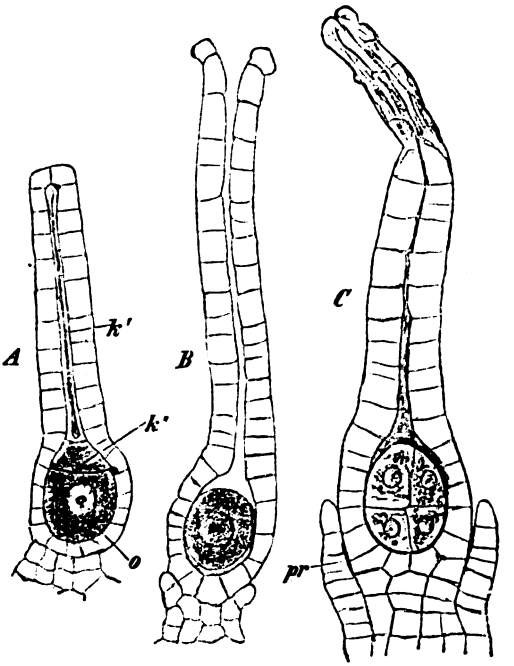


FIG. 95.—*Marchantia polymorpha*. A, young; B, opened archegonium; C, fertilized archegonium after the commencement of the formation of the embryo. k', neck canal-cells; k'', ventral canal-cell; o, egg [-cell]; pr, perianthium (×540).

egg-cell becomes fertilized, the neck closes, even after a few hours, by means of a contraction proceeding from above downwards. Keep the preparation, and after twenty-four hours the presence of a cellulose membrane around the fertilized egg [**oospore**] is easy to recognise. In the course of the next few days the thickness of this cellulose wall still increases.

The fertilized archegonia, which we may meet with upon the longitudinal section, show a shrivelled and brown neck, while the egg [oospore] has divided (Fig. 95, *C*). Around the base of the archegonium, from its foot, a cup-shaped sheath, the so-called **perianthium** (*pr*) begins to develop. This soon encloses the entire swollen archegonium. Upon longitudinal sections of receptacles, which have already spread out their radiating ribs, we see the bright green, swollen archegonia, with base broadened to correspond, situated upon the surface of the receptacle, and decorated at the apex by the remnant of the neck.—From the fertilized egg gradually proceeds the **sporogonium**, which we ultimately see in longitudinal sections, prepared from still older receptacles. The sporogone consists in a shortly-stalked, oval, yellowish-green capsule. The wall of this capsule is unilamellar; if we spread it out with needles, and examine it with stronger magnification, the characteristic thickening rings in the otherwise thin-walled cells will appear. The yellow-walled **spores** are finely pitted. Between them lie narrow, long cells, tapering at both ends, and distinguished each by two brown spiral bands on its wall; these are the **elaters**. The interior of the capsule is filled exclusively with spores and elaters. In capsules already opened (dehisced), we can see that this opening takes place by means of a number of recurved teeth. The elaters are strongly hygroscopic, bend to and fro with changes in moisture of the atmosphere, and so assist the dissemination of the spores.—The sexual organs are not raised upon special receptacles in all the Marchantiaceæ, and in other Liverworts this appearance is altogether wanting. On the other hand, the stalk of the sporogonium in many cases elongates considerably, and carries up the capsule with the spores, which assists the dissemination of the spores.

The **antheridia** of the leaf-bearing **Mosses** are best examined in a genus which has striking male "flowers." We choose a representative of the genus *Mnium*, to wit the widely-distributed *Mnium hornum*, which in May and June "flowers" very freely, and bears female "flowers" and sporogonia at the same time. The

male flowers are, it is true, much more striking than the female, and it is often necessary to search longer for these latter. The male flowers are dark-green, disk-shaped, surrounded by a rosette of leaves, the so-called **perichætium** or **perigonium**. Towards the interior of the flower these leaves decrease rapidly in size. In the axils of the outer, but chiefly, however, of the inner, perichætial leaves, stand numerous **antheridia**, and **paraphyses**, which, moreover, spread over the entire apex of the axis. This is easily shown by median longitudinal sections of the flower, which are best prepared between the fingers, turning the apex downwards in cutting. On these longitudinal sections we see that the flower-axis broadens, after the fashion of a floral receptacle, at the place of insertion of the sexual organs, and in the middle is even a little hollowed. The central conducting bundle, peculiar to species of *Mnium*, has undergone a corresponding broadening, and ends in a chlorophyll-containing tissue, which spreads out under the receptacle. The antheridia and the paraphyses are at once recognised as such, and their structure easy to understand [see Fig. 95A]. The antheridia are club-shaped, shortly stalked bodies, somewhat tapering at both ends. The cells of their wall contain numerous chlorophyll-grains. Where the longitudinal section has opened an antheridium, we see that its wall is composed of a single layer of cells. The contents of the antheridium consist of small, colourless cells, the partition walls of which in young stages of development clearly show rectangular arrangement. The extruded contents of older antheridia opened by the section prove to be composed of rounded cells, still "glued" together, the mother-cells of the spermatozoids, in which the thread-like body of the spermatozoid is already often recognisable. The chlorophyll-grains at the apex of ripening antheridia assume a somewhat brownish tone. Emptied antheridia are open at their apex. The paraphyses are simple cell-rows, the cells of which gradually enlarge upwards, when they are, however, at least the uppermost, again tapering; hence the uppermost cell is always pointed. The walls of the cells are often browned in the lower part of, and not infrequently even higher up upon, the paraphyses; they contain chlorophyll. Cross-sections through the lower part of the flower show in an instructive manner the distribution of the antheridia, their relations with the perichætial leaves and the paraphyses, and also provide us with numerous cross-sections through the antheridia.

Still more striking than the male flowers of *Mnium* are the red-coloured ones of species of *Polytrichum*, likewise found in May and June. For examination we choose *Polytrichum juniperinum*. The outer leaves forming the perichætium, beyond their colour, differ from the ordinary leaves also, in that their unilamellar sheathing portion is continued up to the apex of the leaf. The green *lamellæ*,* characteristic of the genus *Polytrichum*, are found only towards the end or apical portion of the leaf, and almost always confined only to the midrib. On the rapidly-decreasing reddish-brown perichætial leaves, near the interior of the flower, the green lamellæ are developed only on the outermost, sharply outward-bent points. The leaf thus appears ultimately reduced almost to its sheath-portion alone. The antheridia and paraphyses stand in the axils of the perichætial leaves. The middle of the flower is, however, occupied by a vegetative bud, into which the central string of the stem is continued. Thence comes the later growth through the male flowers [**proliferation**], which is normal for *Polytrichum*. The antheridia have the same structure as in *Mnium*. The paraphyses, forming in their lower part a long cell-row, usually broaden at their tip into a spathulate unilamellar cell-surface. If a male flower of *Polytrichum* is squeezed somewhat between the fingers, the contents of the antheridia come out as a milky slime, clearly visible against the reddish ground.

The form of the antheridium of Mosses varies very little, and the accompanying Fig. 95A of that of a moss especially common upon shaded cinder-paths and other places where the substratum has been burnt,

[FIG. 95A.—*Funaria hygrometrica*. *A*, an antheridium bursting; the body of the antheridium shows its wall of cells containing chlorophyll-grains; *a*, the antherozoids (spermatozoids) (× 350). *B*, the spermatozoids more strongly magnified; *b*, in the mother-cell; *c*, free antherozoid of *Polytrichum* (× 800). From Prantl.]

* The leaves of *Polytrichum*, though really unilamellar, like those of other mosses, are rendered opaque by being more or less covered by vertical green scales, or lamellæ, produced upon their upper side. In *P. juniperinum*, each foliage leaf shows about forty-eight such lamellæ, running, as usual, longitudinally, and from 4 to 6 cells long. [ED.]

as well as on old walls, viz. *Funaria hygrometrica*, will serve to illustrate it.

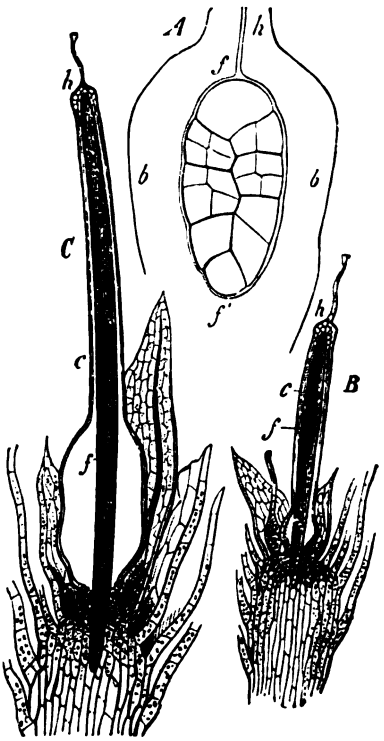
The female flowers of *Mnium hornum*, however, are throughout not so visible as the male, and it is often necessary to seek for them longer. The plants bearing them are far shorter than the male, and somewhat darker in foliage. The upper leaves close together, after the fashion of a bud, in order to protect the female sexual organs, the **archegonia**. As is shown by median longitudinal sections, the apex of the flowering axis is not broadened to any extent, but greatly blunted, and from this we can at once assume that we have to do with a female flower, even if we do not happen at once to find the archegonia. The central conducting bundle of the stem is somewhat swollen under the receptacle, and ends, just as under the male flower, in a chlorophyll-containing tissue. The modified leaves which form the female **perigynium** (equivalent to male perichætium, or, if surrounding hermaphrodite flowers, the **perigamium**), while remaining leaf-like, decrease in size towards the middle of the flower; the apex of the flower is occupied by only a few archegonia, so that it is necessary to take strictly median sections in order to disclose the archegonia. The archegonia are constructed essentially like those of the Liverworts [see Fig. 95B], but the foot-portion is far more strongly developed, only tapering a little downwards, and forms the greater part of the lower half of the archegonium. On these grounds the egg-cell, or **oosphere**, appears comparatively small. We must look for it close under the commencement of the neck, which here appears only a little narrower than the ventral portion. The chlorophyll contents of the cells make the archegonium anything but transparent, and hence the oosphere and the canal-cells of the neck usually need addition of potash to make them visible. In the axils of the perigynial leaves stand numerous **paraphyses**. Each consists of a row of short cells, swelling somewhat upwards. The lowermost cells of these paraphyses have often become brown.

To illustrate the general structure of the archegonium of the mosses, I here introduce Fig. 95B, showing its form and relations with the perichætium in *Funaria hygrometrica*.

Fertilization in the Mosses takes place in all essentials as in the Liverworts, already described. The **sporogonium**, the so-called "moss-fruit," the study of which we will carry on upon the same *Mnium hornum*, consists of stalk or **seta**, and **capsule**. The base of the seta is sunk in the tissue of the mother-plant.

The result of fertilization is here, therefore, somewhat different to that in the Liverworts, and needs a few words of explanation, further illustrated by Fig. 95c. After fertilization, the **oospore** develops into an embryo, an early stage of which is shown in Fig. 95c, *A*. This embryo develops in length both upwards and down-

[Fig. 95B.—*Funaria hygrometrica*. *A*, longitudinal section of the summit of a weak female plant (× 100); *a*, archegonia; *b*, leaves. *B*, an archegonium (× 550), ventral portion with the oosphere; *h*, neck; *m*, mouth still closed; the cells of the axial row are beginning to be converted into mucilage. *C*, the part near the mouth of the neck of a fertilized archegonium, with dark-red cell-walls. (From Prantl, after Sachs.)]



[Fig. 95C.—*A*, origin of the sporogonium (*ff'*) in the ventral portion, (*b b*) of the archegonium, seen in longitudinal section (× 500). *B*, *C*, different further stages of development of the sporogonium (*f*), and of the calyptra (*c*); *h*, neck of the archegonium (× about 40). (From Prantl.)]

wards; downwards it grows into a foot which, as the base of the seta, passes through the tissue of the foot or stalk of the archegonium, and plunges into that of the apex of the moss-stem. (See Fig. 95c, *B* and *C*). Upwardly, the embryo develops into the

capsule, to be hereafter described. The seta remains for a long time short. Accompanying the increase in length, and likewise in thickness, of the young sporogonium, the body of the archegonium, which had enclosed the oosphere, also undergoes further development, keeping pace with the sporogonium in its growth, so as continuously to cover it. The upper part of the neck shrivels, as shown in Fig. 95c, *B* and *C*, at the top. When, later in the development of the sporogonium, the seta rapidly elongates, the body of the archegonium, etc., is ruptured round its base, and is carried upwards, covering the capsule as with a cap,—the **calyptra**. This calyptra, proceeding from the enlarged archegonium, which covers the growing capsule, is in *Mnium* early cast off, so that it is usually difficult to find. It is split up one side to its narrowed apex, and is composed of one, and in part also two layers of elongated cells. The narrowed apex ends in a brown point, which indicates the neck of the archegonium. At the base, where it was ruptured by the growing sporogone, it appears as if cut off. The apex of the capsule, denuded of its calyptra, has a cover or lid [**operculum**] provided with a short beak. With a needle it can be easily loosed, when the edge of the capsular urn, fringed with its teeth, comes to view. The teeth form the **peristome**, the form of which is an important feature in the delimitation of genera, as it is characteristic in each group. The upper part of the seta, passing into the capsule, is called the **apophysis**. In the present case this last is separated from the capsule by a very slight constriction, and is distinguished from it by its brown colour. In some mosses, the apophysis is far wider than the capsule.

In order next to learn the structure of the peristome, we take a cross-section through the capsule, close under the brim of the urn, lift it up, and place it, with the teeth turned upwards, upon an object-slide. We remove the mirror from the microscope, or turn the diaphragm so that no aperture lies under, and observe the object with direct light. In this we need use only a low power. We can decide that the teeth are inserted in the inner brim, that they are wedge-shaped, and cross striate. If we breathe lightly on the object while still looking at it, we shall see the teeth curve together inwards. They are hygroscopic; in damp weather they bend inwards, and so close the open capsule, while in dry weather they bend outwards, and again open the capsule. We count sixteen teeth on the urn. We now lay the same section in a drop of water, and, tearing it through on one side with the

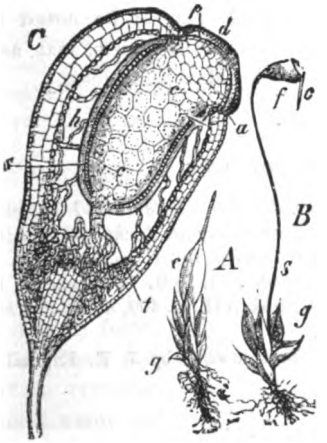
needles, spread it out flat, cover it with a cover-glass, and observe it by transmitted light, and first from its outer side. We then notice, quite at the edge of the urn, a double layer of obliquely-arranged cells, papillately prolonged, pretty strongly thickened, and containing abundant chlorophyll-grains. These cells have colourless walls, browned only at their very base, and there they are very easily disconnected from the edge of the urn, remaining, however, connected together. By means of these cells, the separation of the operculum (lid) is effected; they form the so-called **annulus** at the rim of the capsule. Now laying it with the inner side upwards, the preparation shows us that the cross-striæ already noticed on the teeth are ridges projecting from their inner surface. Besides the outer peristome formed by the teeth, an inner one is also present; it consists of the so-called **cilia**. *Mnium hornum* has, therefore, a double peristome, while there are Mosses with only one, and also without any such peristome. The cilia, like the teeth, are here flat lamellæ, which in their lower part appear divided into chambers, and in their upper part cross-striate, by slight projecting ridges on their inner surface. In the lower part they are fused together into a continuous membrane, which, between each pair of teeth of the outer peristome, is a little bulged. Two cilia stand between each pair of teeth, and present themselves obliquely from the corner. Their edges, the outer in their entire height, the inner only in the upper part, are fringed with small serrate projections. In these the cross-ridges of the surface of the cilia end. Through these serrations the pair of cilia in their upper part are combined by the outer edge, and finally the two fuse into a single narrow, elongated apex. With these pairs of cilia alternate very small ones, which, from three to five in number, stand in front of the teeth of the outer peristome. A delicate cross-section, taken somewhat deeper through the capsule, shows in the interior of this the column formed of large-celled tissue, the **columella**. Around this columella lies the cavity filled with spores. The inner wall of this is formed by the columella itself, the outer by a layer of tissue, usually two cells thick, and containing chlorophyll, which appears separated from the wall of the capsule by a very loose chlorophyll-containing tissue. The **wall** of the capsule consists of two or three layers, and is covered by a sharply-defined **epidermis**. The cells of this latter are more strongly thickened on their outer walls. The **spores** contain chlorophyll-grains, their wall is brownish, and studded with fine

warts; in favourable cases a three-sided pyramidal tapering of one side of the spore can be noticed. This tapering arises from the tetrahedral position of the spores inside their mother-cell; it indicates the contact surfaces of its three sister-spores.*—A perfectly median longitudinal section, which we prepare from a capsule which is still green, and provided with its lid [operculum] but is already fully formed, shows us uppermost the lid, consisting externally of one sheath of browner, strongly-thickened cells, and internally of many layers of thin-walled cells. At the limits between lid and urn lies the double layer of the obliquely-arranged, chlorophyll-containing cells, already known to us, by which the separation of the lid is effected. The brown cells, which adjoin the urn below, are distinguished by their small height. Similar cells adjoin these small ones towards the interior, and form thus an inward projecting ledge of thickened, brown cells, on which are set the teeth of the outer peristome. About the thickness of a cell removed arise the cilia. As the history of their development teaches us, these teeth and cilia arise by local thickening of the opposite walls of one and the same layer of cells adjoining the inside of the lid. The teeth proceed from definite portions of the outer walls, connected in the ascending direction; their cross-ridges indicate inner adjoining cross-walls, upon which the thickening has continued for some little distance. The cilia proceed from the thickened parts of the inner walls of this same layer of cells, and bear slight ridges at the places of junction of the next inner partition walls.

In our median longitudinal section the lid is hollow; the inner tissue, after the formation of the teeth and cilia, has shrivelled up, separating from the inner surface of the cilia, which extend to the top of the lid. This tissue now forms on the columella only a projecting conical knob. The **columella** is visible in its entire length; similarly we can survey the **spore-sac**, its outer wall, the looser tissue lying between this and the wall of the capsule, and lastly this wall. The spore-sac, so long as the lid has not been cast off, is closed above by a thin layer of tissue. Later on, it opens by the tearing of this layer. At the base of the capsule, under the spore-sac, an annular cavity has been formed. The **apophysis**, as is now seen, is provided with **stomata**, for on well-nigh every median longitudinal section, such will be cut. They lie

* The contents of the spore mother-cell divide into four, which are situated as if at the four corners of a tetrahedron. [Ed.]

below the level of the epidermis; a pit leads down to each; an air-chamber adjoins it internally. It is surrounded by chlorophyll, containing tissue, the intercellular spaces of which communicate with the annular cavity under the spore-sac, and with the intercellular spaces of the entire chlorophyll-containing tissue separating the wall of the capsule from the spore-sac. All the stomata are cut in the direction of their length, and give figures which, so far as can here be determined, agree with those of the Vascular Cryptogams, and of Phanerogams. This latter is so much the more striking since the apophysis (or, in other cases, the wall of the capsule as well) is the only place in mosses where true stomata, constructed after the type of the higher plants, are borne.—In



[Fig. 95D.—*Funaria hygrometrica*. *A*, a young leaf-bearing plant (*g*), with the calyptra (*c*). *B*, a plant (*g*) with nearly ripe sporogonium; *s*, its seta; *f*, the capsule; *c*, the calyptra. *C*, longitudinal section of the capsule bisecting it symmetrically; *d*, the operculum; *a*, the annulus; *p*, the peristome; *c*, *c*, the columella; *h*, the air-cavity (which here extends around as well as below the spore-sac); *s*, the spore-layer, consisting of the primary mother-cells of the spores (× about 20). (From Prantl.)]

[Fig. 95E. Mouth of the capsule of *Fontinalis antipyretica*, the operculum having fallen off. *ap*, outer peristome of teeth; *ip*, inner peristome of cilia (× 54). (From Prantl.)]

order to complete the impression we have obtained, let us now examine sections of the surface of the capsule and of the apophysis. We can decide that on the surface of the capsule, stomata are wanting; between the brown-walled cells of the apophysis we see, however, pits which lead up to the stomata. If we turn the section over, and examine it from the inner side, we can in favourable cases distinguish the two **guard-cells** of the stomata, formed as in higher plants. Upon such sections we can at the same time determine that the green cells between the wall of the capsule and the

spore-sac are joined together in longitudinal direction, that they are branched, and have all the aspect of algal threads. Moreover, on cross-sections through the apophysis stomata have usually been cut, the two guard-cells of which are not difficult to see. At the seta, the differentiation of the epidermis ceases; its surface is occupied by two or three layers of yellow to reddish-brown strongly-thickened cells, the cavities (lumina) of which, passing inwardly, become gradually larger. In the interior of the seta a central conducting bundle is differentiated. Median longitudinal sections taken near the apophysis show that these relations, beginning close to this region, are stamped upon the seta quite gradually.*

[The accompanying Figures 95D and 95E will serve to render more easy the comprehension of the foregoing description of the structure of the capsule in the mosses. It should be noted that the author's descriptions are confined to the Bryaceæ, just as in the Liverworts they were confined to the Marchantiaceæ.]

NOTES TO CHAPTER XXV.

[1] Goebel, *Die Muscineen* in Schenk's *Handbuch der Botanik*, Bd. II., p. 338.

[2] Compare, A. Zimmermann, *Ueber die Einwirkung des Lichtes auf den Marchantienthallus. Arb. aus d. Bot. Inst. in Würzburg*, Bd. II., p. 665.

[3] Leitgeb, *Untersuchungen über die Lebermoose*, VI. Heft, 1881, pp. 20, 117; Goebel, *l.c.*; Strasburger, *Jahrb. f. wiss. Botanik*, VII., p. 409, and *Befruchtung und Zelltheilung*, 1877, p. 12.

N.B.—A recent work on "Mosses," for beginners, is by J. E. Bagnall (1*s.*, Sonnenschein & Co.).

[*Note to page* 274.]

* The cilia can be seen with especial clearness in dry preparations, which are obtained by allowing a fresh preparation, or one which has been fixed in any way, to dry slowly and perfectly without covering it with a cover-glass. Such preparations can then be covered with a cover-glass, and can be closed in any suitable way and preserved.

* The stomata upon the apophysis of *Funaria hygrometrica* are of interest in that the division of the mother-cell, so as to form the guard-cells, is incomplete. Hence there is but one guard-cell, shaped like a ring, with a short median cleft. In the early stages of development the dividing wall was complete, but its ends are subsequently resorbed.

CHAPTER XXVI.

THE REPRODUCTION OF THE VASCULAR CRYPTOGAMS.

MATERIAL WANTED.

Fertile fronds of *Scolopendrium vulgare*, the Hart's-tongue fern. Fresh. (Alcohol material in part answers.)
The same of the Male Fern (*Aspidium Filix-mas*).
The same of the common Polypody (*Polypodium vulgare*).
Fresh spores of *Ceratopteris thalictroides*.
Prothallia of *Polypodium vulgare*. Fresh.
Fructifying plant of *Selaginella Martensii*. Fresh, or dried.

THE **sporangia** of Ferns stand, with few exceptions, on the under side of the leaves. They usually form groups, which are called **sori**. The whole sorus is commonly covered by a strong outgrowth of the leaf, the **indusium**. The indusium can be very variously developed. If the edge of the leaf turns over the sorus, we speak of it as a **false indusium**.—As an example for investigation, we select the common Hart's-tongue fern, *Scolopendrium vulgare*. The leaf is traversed by a strong midrib, from which arise weak lateral veins, only slightly inclined forwards. In the upper half or along the greater part of the length, of the fertile leaf the sori are formed. They retain the same direction with the lateral veins. Externally they appear more or less completely covered by two, at first, overlapping lip-like indusia, which later are more widely separated and spread open. It is only necessary to prepare a delicate cross-section of a piece of a fertile leaf. For this purpose we select a leaf on which the sori are already brown, but the edges of the indusium have not yet spread open. We cut with the scissors a narrow strip out of the leaf, parallel with the sorus, clamp this strip between pieces of elder-pith, or pack several such strips together, one behind the other, in which case no elder-pith is needed, and take delicate cross-sections through them. The cross-section (Fig. 96, *A*) through the tissue of the leaf shows us an **epidermis** on the upper and under side, and a

spongy-parenchyma, the cells of which lie more densely together under the upper epidermis. There is no palisade layer. The apparently simple linear sori now appear divided into two. These stand right and left, inclined to one another, each in the angle between the leaf-surface and an indusium, and each close over a **fibro-vasal bundle**. The surface of the leaf at the places in question is hollowed into a furrow, and between the two sori rises

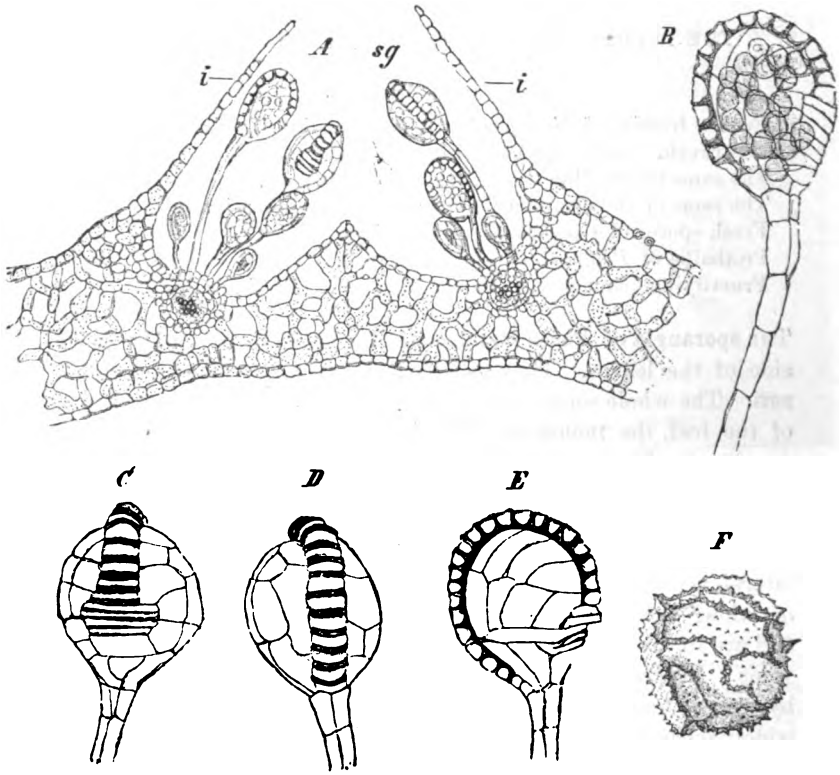


FIG. 96.—*Scolopendrium vulgare*. *A*, cross-section through the fertile part of the leaf; *i*, indusium; *sg*, sporangium. *B–E*, sporangia; *B* and *E*, seen from the flanks; *D*, from the dorsal side; *C*, from the ventral side; *F*, a spore. (*A*, × 50; *B–E*, × 145; *F*, × 540.)

into a ridge. The epidermis at the base of the furrow, studded with sporangia, impinges immediately upon the **bundle-sheath**. The epidermis of the under side of the leaf, and of the furrows, unite in order to pass over into the **indusium** (*i*). This begins, therefore, with a double layer of cells, which quickly passes over

into a single one. This layer of cells has the structure of the neighbouring epidermis, except that it is wanting in stomata and chlorophyll-grains. Yet it contains smaller colourless chromatophores. From the base of the furrow arise the **sporangia** (*sg*); they can be seen in different stages of development; each derives its origin from a single epidermal cell. Even with weak magnification (Fig. 96, *A*) we can distinguish in each sporangium a **stalk** and a **capsule**, and on older a yellow-brown ring, the **annulus**, can be noticed on the capsule.—For further study we make use of somewhat stronger magnification (Fig. 96, *B*). The stalk passes over from a single to a double row of cells. The capsule has a unilamellar **wall** of cells. As is shown by different views of the wall of the capsule (*B–E*) the annulus is composed of a row of cells, of this capsule-wall, which project outwards. These cells form a row, which, commencing at the stalk, passes over the apex, and down the opposite side, and, flattening and becoming broader, dies away without again reaching the stalk. The inner and tranverse walls of the cells of the ring are strongly thickened and browned; the thickening decreases in the transverse walls in the direction of the outer surface. The sporangium opens between the broad cells in which the ring ends (Fig. 96, *C*, *E*); the one half of these broad cells then lies on the one, the other half on the opposite side of the fissure. The cause of the rupture lies in the ring, which in drying tends to diminish its curvature. The brown wall of the ripe **spore** shows a beautiful structure (Fig. *F*). It is covered on its outer surface with a network of cockscomb-like projections.—In *Aspidium Filix-mas*, the "male Fern," we find indusia, in shape between a heart and a kidney, which with age become leaden-coloured, and finally brownish, shrivel somewhat, and no longer completely cover the dark-brown sori. The sporangia have almost the same structure as those of *Scolopendrium*. Upon some of them we see a short **glandular hair**, ending in a unicellular head, arise from the stalk. The sporangia are attached to a cushion-like prominence, a **placenta**, which lies over a fibro-vasal bundle. To this latter adjoins reticulately thickened tracheïdes, which are distributed in the placenta. At its apex the placenta bears the indusium, inserted by being curved down into the form of a stalk.—If we take a preparation in water which includes sporangia that are ripe, but still closed, and run in from the edge of the cover-glass a water-withdrawing medium, best glycerine, the sporangia slowly open before our eyes. The

annulus ultimately becomes strongly concave. Then follows, with a jerk, an opposite movement, which more or less completely closes the sporangium. The entire phenomenon can in lessened degree be repeated once or several times. Careful observation shows that during the dehiscence the outer walls of the annulus project strongly into their cells. The closing movement corresponds with the movement in which, inside the cells of the annulus, at the maximum of loss of water, an air-bubble is separated out in each cell. If gas has not come out in every cell, the outward curvature still continues in those in which this has not happened, which occasions the secondary movements of dehiscence. If now the glycerine is replaced by water, the air-bubble in each cell decreases in size, and ultimately disappears, being resorbed by the cell-sap, while the sporangium almost completely closes. With renewed addition of glycerine, the reverse phenomenon can again be produced.—It may be of interest to us also to turn our attention to the **naked sori** of *Polypodium vulgare*, the common Polypody fern. The sori are entirely without indusia, and each one lies over the end of a fibro-vasal bundle. The placenta scarcely projects above the surface of the leaf. The sporangia are constructed upon the same type as in the foregoing species.

We select the Ferns likewise for the purpose of studying the structure of the sexual organs, and of following the processes of fertilization, in the group of Vascular Cryptogams. The *prothallus*, which is the first and sexual generation of Ferns, is always easy to produce. We obtain them by sowing the spores, or else collect fertile prothallia. In this we will confine ourselves to the family of Polypodiaceæ, the most widely-spread, and by far the richest in species. For sowing, we take the spores of *Ceratopteris thalictroides*, cultivated in all botanical gardens, and therefore easy to procure. If this should not be procurable, the spores of almost any fern will do equally well. If on the other hand we would collect fertile prothallia, those of any species of the Polypodiaceæ will serve for examination. To find prothallia in the open air is attended with considerable difficulty, and we shall therefore do well to look for them in plant-houses. On damp shaded walls, on the stems of tree-ferns, on flower-pots we can almost always find prothallia. On the fibrous peat,[2] much used now in the culture of Orchids, *Sarracenia*, etc., and which is often permeated by *Polypodium vulgare*, are usually found numerous prothallia of this fern, which we will here select for closer

examination. As in most other Polypodiaceæ, the prothallia of the common Polypody fern have the form of small, heart-shaped, bright-green leaves, lying on the substratum. We seize a prothallus of medium size, with the forceps, always taking hold of the place where it is attached to the substratum, and lift it away. We immerse it in water, in which we move it for some time here and there, in order to wash off the fragments of adhering soil, and then lay it, with the ventral side upwards, in a drop of water on the object-slide, and examine it under a cover-glass. The prothallium, as already noted, is heart-shaped. It consists of polygonal cells, containing numerous chlorophyll-bodies. In the anterior indentation lies the small-celled **meristem** of the **growing point**. Only in its central portion is the prothallus multilamellar, as can readily be proved by changing the focus. This median portion is the so-called **cushion**. It passes over at the sides into the unilamellar thallus, and slopes gradually also towards the base of the prothallus. From the after-parts of the prothallus *i.e.*, those furthest from the growing apex, arise the **root-hairs** or **rhizoids**; they are especially produced in the median portion of the prothallus. They are long, unicellular sacs, which soon become brown. At the edge and under side of the prothallus, individual cells, moreover grow out into short, almost without exception, unicellular papillæ, which, like the rhizoids, are cut off by a partition-wall at their base. If we have chosen for investigation comparatively young prothallia, they are male; if we have taken too old ones, they bear exclusively female sexual organs. Between these two are such as bear both sexual organs. The sexual organs, like the root-hairs, stand only on the ventral side of the prothallus. The male sexual organs, **Antheridia**, are found on the hinder parts of the prothallus; they arise between the root-hairs; but also further beyond these laterally. Their formation proceeds in the direction of the apex [acropetally]. They appear as globular arched structures (Fig. 97, *A*), which, in a ripe condition, contain smaller globular cells in greater number inside a unilamellar wall. On the other side [more behind] the ripe antheridia stand those which are already emptied, recognisable by the browning of their inner walls, and showing a stellate gap in their lid-cell. A full insight into the structure of the antheridia is only obtained when we examine them in profile. Such profile views are not seldom obtained in many accidentally-bent parts of the prothallus; we obtain them easily, also, if we suitably bend around with needles

prothallia which are rich in antheridia. In correct side-views (Fig. 97, *A*) we now readily determine that the antheridium is seated upon the middle of a slightly arched prothallium cell (*p*), and cut off from it by a partition membrane. The wall of the antheridium consists, almost without exception, of two stages of lateral cells (1 and 2) and a lid cell (3). The cells of the lower stage have a broader cavity than in the upper stage or the lid. The side-view of an emptied antheridium (Fig. 97, *B*) shows the lateral cells very strongly swollen; they therefore stand out very clearly. The cavity of the antheridium is then correspondingly narrowed, the lid-cell pressed flat, and ruptured. If we now turn again to the surface-view of the prothallium, and observe an emptied antheridium from above, we can determine upon it that the lateral cells are without inner segmentation. Inner partition walls are in no way visible, and we come, therefore, to the conclusion that the wall of the antheridium consists of annular cells. Each stage is therefore formed of but one ring-like cell. The entire wall of the antheridium consists, therefore, of two such superposed ring-like cells, and a lid-cell. Annular cells of this kind are a rare phenomenon,* but constantly recur in the antheridium of the Polypodiaceæ.—In general we should find similarly-constructed antheridia on the prothallia of other Polypodiaceæ. The only common departure from the form here represented, is that in which the antheridium has a lower, flat stalk-cell, and the side wall consists of only one annular cell.—If we have for examination prothallia which have not been wetted for a long time, we shall not have long to wait for the emptying of individual ripe antheridia. The mechanism of the evacuation consists in the pressure which the annular lateral cells bring to bear upon the contents, besides which a swelling substance is developed between the separated internal cells of the antheridium. The lid-cell is

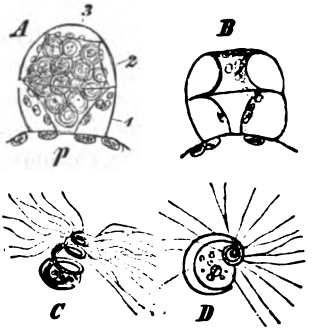


FIG. 97.—*Polypodium vulgare*. *A*, ripe; *B*, emptied antheridium; p, cell of the prothallus; 1 and 2, lateral cells; 3, lid or cover cell. *A* and *B* (×240). *C*, a spermatozoid in movement; *D*, one fixed with iodine solution. *C* and *D* (× 540).

* Annular cells are likewise met with in some Ferns in connection with the development of stomata. [ED.]

ultimately ruptured, and the contents of the antheridium squeezed out, whereon the annular cells increase in size. The contents of the antheridium come out in the form of isolated, globular cells, the **spermatozoidal cells**, which first lie resting for a short time in the surrounding water. In each cell, even with weak magnification, is to be recognised a coiled thread, the **spermatozoid** [**antherozoid**], and a central collection of fine granules. The walls of these cells dissolve in the surrounding water, and even in a few seconds individual spermatozoids begin to free themselves. This occurs with a jerk, whereby the coils of the body of the spermatozoid separate. One spermatozoid after another thus escapes. We follow individuals in the surrounding water, and notice that they progress comparatively rapidly, and at the same time rotate upon their axis. If a condenser is at our disposal, we proceed, by cutting off the direct rays of light by means of the diaphragm, to obtain a dark field of view. In this dark field the spermatozoids swarm about as illuminated objects.* After about 20 to 30 minutes, the movement slackens, and finally ceases. During these last stages of the movement, the form of the spermatozoids is not difficult to recognise. This is more easily attained if to the drop of water containing the spermatozoids is run in a 10 per cent. clear filtered solution of gum arabic, and so the rapidity of their movement is diminished.[3] The spermatozoid (Fig. 97, *C*) is composed of a band, rolled after the fashion of a corkscrew. The turns at the anterior end are narrow, but towards the posterior become broader. The anterior narrow turns bear long fine **cilia**. Between the posterior turns lie fine granules, and we often recognise a "vesicle" or "float" containing them. By the addition of a little potassium-iodide-iodine the spermatozoids are very beautifully fixed.

At the anterior indentation of the prothallus, we see the female sexual organs, the **archegonia**. Nearest the indentation, they are still imperfect; further in, are ripe but unopened; finally, dead and opened, brown inside. The female sexual organs are very easy to distinguish from the male. They project above the surface of the prothallium in the form of short, cylindrical structures, curved away from the anterior indentation. This free portion of the archegonium is only its **neck**, whilst the ventral portion is found sunk in the tissue of the prothallium. At the neck we distinguish

* For further information on this head, see "Dark field illumination" in any handbook on the microscope. [Ed.]

a unilamellar **wall**, formed of four cell-rows, and a central **canal**, the contents of which, in ripe archegonia, appear granular in the central portion, and strongly refractive peripherally. This inner canal, the **neck-canal**, broadens upwards like a club. Below it passes into the **central cell** of the archegonium, in which is found the **oosphere**. This last, it is true, is scarcely distinguishable.—If the prothallia had been allowed to remain dry for several days before the commencement of the investigation, we shall probably be successful in seeing the opening of an archegonium. We choose for continuous observation an archegonium the contents of the canal of which appear strongly refractive. Often the opening results almost instantaneously; often it is necessary to wait some time. The opening of the neck is the result of the pressure which the strongly-refractive swelling substance of the neck-canal exerts upon the wall of the neck. The four cells at the apex of the neck suddenly separate from one another, and the contents of the neck-canal pour out. The strongly-refractive substance of this diffuses as a colourless mucilage in the surrounding water, while the granular contents are gradually disorganized. The evacuation of the contents takes place interruptedly; first come out the contents of the neck-canal, then those of the ventral canal-cell last cut off from the oosphere. Under specially favourable conditions we may now see the entrance of the spermatozoids into the archegonium. The chances of this are increased if we have placed with the older prothallium, selected for the examination of the archegonia, some quite young ones, rich in antheridia. If spermatozoids are diffused in the preparation, we see them, so long as the archegonia are closed, quietly swimming by them. If on the other hand an archegonium has opened, the spermatozoids, from a measurable distance round, take the direction of the mouth of the canal, and are intercepted by the mucilage. Inside this mucilage their movement is slackened, while they retain their original direction; they enter into the neck-canal, and reach the oosphere, into which they are taken up. As has been recently determined, here also the secretion of a substance from the neck of the archegonium takes place, which acts as a chemical stimulus on the spermatozoids, and determines the direction of their movement.[4] The specific stimulant in this case is **malic acid**, which to the extent of about 0·3 per cent. is represented in the mass evacuated from the neck of the archegonium. Thus these spermatozoids can be successfully enticed into capillary tubes, which are fused at one end, and under

the air-pump are injected with a fluid which contains 0·01 to 0·1 per cent. malic acid, combined with any base, just the same as into the neck of the archegonium. The spermatozoids of Ferns swarm into such capillaries, likewise into large hairs, best of all those of the leaves of *Heracleum Sphondylium*, the Hog-weed, or Cow-parsnip, if these are laid, with their ends cut off, in water containing spermatozoids.[5] For the spermatozoids of the mosses, cane-sugar is the specific stimulant, while with *Marchantia*, another, not yet determined, substance proceeds from the archegonium.—It has been experimentally determined[6] that a single spermatozoid suffices for fertilization; but usually several penetrate into the archegonium, of which, however, only one finds admittance. These processes cannot be followed in detail, as the prothallium of *Polypodium* is too opaque; they can be seen much better in *Ceratopteris*. We can, however, state here, that the spermatozoids do not take their posterior vesicle with them into the archegonia, but, so far as they arrive there with it still clinging to them, it is left in the mucilage in front of the opening. Now and again the number of the spermatozoids which arrive is so large that they ultimately bore in between one another, and elongating thread-like, fill up the entire canal of the archegonium, and still form a tuft before its opening.—There still remains one thing, to see the archegonia in sections. These must only be cut median, as the archegonia are found only on the median line of the prothallus. In order to facilitate the cutting, we lay several prothallia, which are carefully arranged, one upon another, after we have previously removed all grains of sand. We now find the desired structures very easily on the sections. The archegonium, as we see (Fig. 98, *A* and *B*), has its ventral portion sunk in the prothallium, the neck being bent. Neck canal-cell (*K'*) and ventral canal-cell

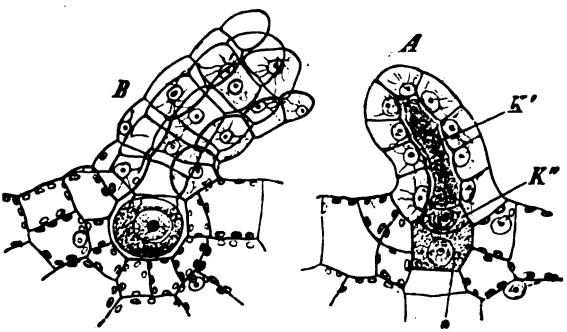


FIG. 98.—*Polypodium vulgare*. *A*, unripe archegonium; *K'*, neck canal-cell; *K''*, ventral canal-cell; *o*, oosphere; *B*, ripe opened archegonium (×240).

(*K''*) are now distinguishable; as also the oosphere (*o*), together with its nucleus. The ventral portion of the archegonium has become covered by a layer of flat cells. In the ripe opened archegonium (*B*) a colourless spot, the receptive spot, can often be noticed at the apex of the oosphere, at which takes place the reception of the spermatozoids. Individual less median sections may show us the antheridia also in profile.

In order to grow prothallia from spores, as of *Ceratopteris* recommended above, we can sow the spores on a piece of moderately soft tile, laid in water in a saucer, or upon a flower-pot or flower-pot saucer similarly kept constantly moist. In a room it may be covered over with a bell-globe. In this way all the early stages of development can be well obtained, and it needs only to scrape off some of the germinating spores day by day with the blade of a pocket-knife, and lay them in water on an object-slide, to be able to follow the development. For full-grown prothallia for section-cutting, the spores can be well sown on a bed of cocoa-nut fibre refuse, flattened down in a large flower-pot saucer, with a hole in the bottom, or a seed-pan, and well drained, kept moist until towards the time they are needed for examination. Overhead watering, if needed, can be given with a spray such as is used for diffusing scent.[a]

The **Selaginelleæ** are **heterosporous** Lycopodineæ; they possess two kinds of sporangia and spores, and we will therefore turn our attention to them, in order to complete the view we have taken of the other Vascular Cryptogams. The Selaginelleæ are also known as the **Ligulatæ**, because their leaves are provided with a small ligule at the base. We will examine more closely the *Selaginella Martensii* (Sprg.), universally distributed in plant-houses. Fertile specimens are easy to recognise by the spikes which they develop on the last branches of usually numerous shoots. The vegetative body of the plant is spread in one plane; it bears four rows of leaves in pairs, which cross one another obliquely. In each pair the upper leaf remains small, the under is considerably larger. The two rows of upper leaves on the dorsal surface press against the stem with their upper surface. The two rows of under leaves on the ventral surface are placed laterally, flatly spread out, with their upper surface above. The vegetative body of the plant is therefore **bilateral** and **dorsi-ventral**; that is, it admits only one plane of symmetry, which divides the body into a right and left half, and exhibits a ventral and dorsal surface. The fertile terminal

[a] See note on page 297*a*.

spikes, on the other hand, are quadrangular, provided with four rows of symmetrically-arranged leaves, directed outwards.—We next inform ourselves as to the structure of the spike, by pulling off one leaf after the other with needles under the simple microscope, beginning at the base. We see an ovate, somewhat flattened **sporangium** stand in the axil of each leaf. Even in this operation we shall have noticed that many sporangia are larger, and show projecting bosses. If we open the large, bossed sporangia with the needles, four large spores will come into view, which completely filled the sporangium, and arched its wall out locally; if we open a small sporangium, this proves to be filled with numerous small spores. The large sporangia are female sporangia (**macrosporangia**), the large spores female spores (**macrospores**); the small sporangia and spores are male, and are distinguished as **microsporangia** and **microspores**. The small spores are triangularly pointed on one side, with reticulate markings, and usually hang together in tetrads. The same relations, increasing in accordance with size, are met with on the four macrospores. We see clearly upon them the triangular tapering of one side; in order, on the other hand, to be able to distinguish well the reticulately connected ridges on the cell-wall, it is desirable to crush the spores. The walls of the microspores soon become dark brown, while the macrospores remain far clearer. If we examine the leaves, from which we have removed the sporangia, we see the **ligule** arise close under the place of insertion of the removed sporangium, as a tongue-shaped membrane. A further removal of leaves from the spike shows us that the macrosporangia are far scarcer upon it than the microsporangia, and always appear preponderatingly on the lower parts of the spike. The ripe sporangia dehisce transversely into two valves.

In conclusion, it may be mentioned that the Selaginelleæ, in drying, preserve so excellently, that we can use softened herbarium specimens in order to study the growing point and the origin of the sporangia. Sections through fresh material, as well as material thus softened, can be made very transparent with potash-solution.

NOTES TO CHAPTER XXVI.

[1] Compare Leclerc du Sablon, *Ann. des Sci. Nat. Bot.*, VII. Ser., vol. ii., p. 10, 1885.

[2] *Terre fibreuse* of the Belgian nurserymen.

[3] Compare Pfeffer, *Unters. a. d. Bot. Inst. zu Tubingen*, Bd. I., p. 370.

[4] The same, p. 360. [5] The same, p. 410.

[6] Strasburger, *Jahrb. f. wiss. Botanik.*, Bd. VII., p. 405.

[*Note to page* 296.]

* As the raising of fern prothallia from spores has many points of interest, a few additional observations upon it may be made here. Amongst the quickest of all spores to germinate are those of the Royal Fern, *Osmunda regalis*. The spores of *Ceratopteris thalictroides*, recommended in the text, are of special utility if obtainable, owing to the transparency of the structures developed from them. A highly recommended method is to sow the spores upon a slab of peat or turf, which has been first boiled in water in order to kill any seeds or spores it may contain, and then soaked with the culture fluid recommended for the cultivation of *Spirogyra*. The spores are then sown, and the turf covered with a bell-glass and placed in a north window. With a favourable temperature germination begins in from 3 to 5 days.—With care to keep them moist, the spores can likewise be germinated upon a glass slide, and, with the very careful addition of minute quantities of culture-fluid, can be grown to some size, though their growth thus is usually much more slow. Owing to their large size, the spores of *Ceratopteris* lend themselves pretty readily to this mode of culture, which enables easy observation under the microscope, and facilitates also the fixing and permanent preservation of specimens showing the early stages of germination and prothallial development.—The spores of *Ceratopteris* can likewise be germinated by sowing upon a nutrient fluid, and preserving a moist atmosphere. Floating prothallia are then developed, with rhizoids and sexual structures in their normal position, but submerged.

CHAPTER XXVII.

THE REPRODUCTION OF GYMNOSPERMS.

Material Wanted.

Male flowers of *Pinus* (*e.g.*, the Scotch Fir, *P. sylvestris*). Best in alcohol. March or April.

Male flowers of the Yew (*Taxus baccata*). Fresh, or in alcohol. March.

Fertilized ovules of Yew. Fresh, or in alcohol. End of April.

Young female cones of the Scotch Fir (or other *Pinus*). Fresh, or in alcohol. End of May.

Cones of the Red Fir (*Picea vulgaris*, Lk.). Fresh, or best in alcohol. Mid-June.

Seeds of the same. October.

Phanerogamic plants fall into the two great divisions of **naked seeded**, or **Gymnosperms**, and **enclosed seeded**, or **Angiosperms**. These divisions are especially distinguished by the structure of the flower and the processes of fertilization and embryology, which we will first examine in the Gymnosperms. We first make ourselves acquainted with the structure of the **male flower** [1] of the Scotch Fir (*Pinus sylvestris*). This plant flowers in May or June, according to the district ; but it can be investigated very well in alcohol material, which, because too brittle, should be laid, at least one day before the commencement of the investigation, in a mixture of equal parts of alcohol and glycerine. Material thus prepared can be cut much better than if fresh.—We first make out that the male flowers here stand in large numbers on the lower parts of a shoot of the same year. They are arranged according to a $\frac{2}{5}$ phyllotaxy, and correspond in their arrangement exactly to the condensed shoots, each bearing two needle-leaves, which succeed the flowers in uninterrupted series. The flowers also, like the condensed leafy-shoots, stand in the axils of scale-leaves. Upon the stalk of the male-flowers, we find first three decussating pairs of bracts. The lowermost pair is placed laterally with regard to the

mother axis, an arrangement which is due to the necessities of space, and which recurs almost without exception with the first pair of leaves of the vegetative buds of Gymnosperms. To the bracts of the short flower-stalk succeed the **stamens**, closely crowded, usually arranged in ten vertical rows. The floral axis is elongated, fusiform. A single stamen separated and examined under the simple microscope appears circular; its under-side is occupied by two longitudinally-inserted **pollen-sacs**, touching one another in the middle line; at its apex running out into a short, outwardly-directed border. Median longitudinal sections through

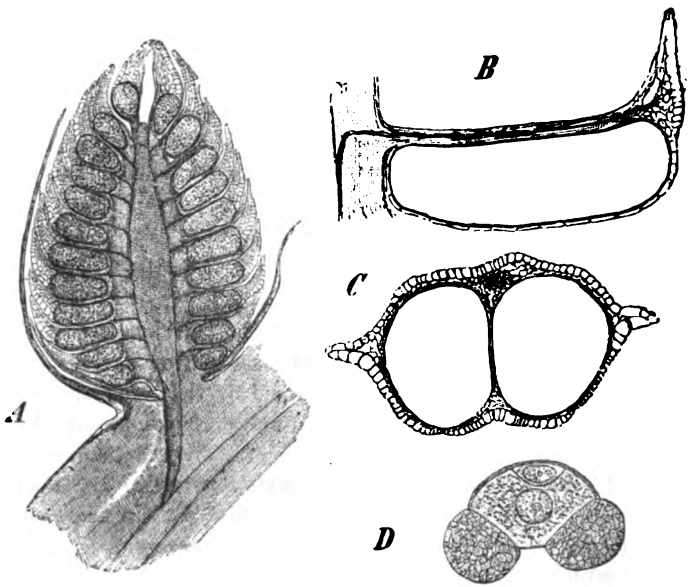


FIG. 99.—*Pinus Pumilio*, resembling *P. sylvestris*. *D*, from *P. sylvestris*. *A*, longitudinal section through a nearly ripe male flower (× 10). *B*, longitudinal section through a single staminal leaf (× 20). *C*, cross-section through a staminal leaf (× 27). *D*, a ripe Pollen-grain (× 400).

the flower, shortly before the dehiscence of the anthers (Fig. 99, *A*), show, especially after treatment with potash, the course of the fibro-vasal bundles in the floral axis, the series of staminal leaves, each with a single fibro-vasal bundle, the insertion of the pollen-sacs on the staminal leaves. Upon less complete longitudinal sections, thinner spots can be readily found, in which the structure of the individual staminal leaf (*B*) is followed still better.

We then prepare tangential longitudinal sections through the flower, in order to obtain cross-sections of single staminal leaves, and pick out such an one for closer study (*C*). We see that the two pollen-sacs adjoin in the middle line, and, when perfect, are usually separated only by a flat wall of collapsed cells, in the middle of which may be interposed one or more layers of flat starch-containing cells. Upon their free outer surface the pollen-sacs are covered by the epidermis, to which, towards the interior, usually only collapsed cells adjoin; towards the dorsal surface of the leaf likewise the anther cavities are closed in the same way. In the median line of the staminal leaf, above and below the partition wall separating the two pollen-sacs, runs a strip of **mesophyll**. The upper is thicker, and is traversed by the very delicate fibro-vasal bundle. At the two side-edges of the staminal leaf, the epidermis projects into a weak or more strongly-developed wing; in the latter case, a little mesophyll can be found between the two layers of epidermis (*C*). On the under side of the pollen-sacs the epidermal cells diminish in size from both sides; at the places of weakest development, the pollen sacs open. These pollen-sacs closely resemble the sporangia of Lycopodiaceæ; researches in comparative development have, in fact, led to the conception that the **pollen-sacs** of Phanerogams, and the **microsporangia** of Cryptogams are homologous structures.—If we look now to the **pollen-grains** developed in the pollen-sacs, where possible in the fresh state, we shall note that each of these consists of a central body, upon which are placed laterally two vesicles (*D*). If the flower is ripe, the two vesicles appear dark, because filled with air. They show delicate markings upon their surface. The interior of the central, true pollen-grain, contains finely granular protoplasm, and a large nucleus. Shortly before dehiscence—*i.e.*, before the opening of the pollen-sacs—a division takes place in the pollen-grain, by means of a convex partition wall (*D*), which limits a lenticular cell on that side of the pollen-grain which is turned away from the place of insertion of the vesicles. This cell is best seen when the pollen-grain, as in our figure, lies on its side. An exactly similar cell is also cut off from the **microspores** of the heterosporous Lycopodiaceæ, before the commencement of the stages of development which lead to the formation of the antherozoidal cells. In both cases alike we can distinguish these cells as **vegetative cells**. The wings (vesicles) of the pollen-grain arise, as the story of their development shows,

rather late, and by the upheaval of the cuticle, between which on the one hand, and the inner thickening layers of the wall on the other hand, a watery fluid collects.

From the structure of the male flower of *Pinus sylvestris* examined above, the male flower of *Taxus baccata* (the Yew) differs most. This flowers somewhere in March, but by means of alcohol material we can be independent of time. The male flowers of *Taxus* stand in the axils of leaves on the previous year's twigs. They commence with some decussating pairs of scales, and pass over into scales arranged on a $\frac{1}{5}$ phyllotaxy. The scales become successively larger, and at length follow, in quite indefinite arrangement, the shield-shaped staminal leaves upon the elongated axis. These, as examination with the lens will at once show, have a by no means slight resemblance to the fertile sporangiferous leaves of the spikes or cones of *Equisetum*. If we remove a staminal leaf with the scalpel, and examine it under the simple microscope, we shall find from five to seven pollen-sacs inserted on the inner side of the shield and its stalk. These are mounted on the shield with their base, on the stalk with their inner side. Laterally, towards one another, they are mostly free, and quite free on their outer surface and at their apex. We can fully inform ourselves on this point, if we further bring median and tangential longitudinal sections to our aid. The former show the staminal leaves and pollen-sacs in longitudinal section, the latter in cross-section. In longitudinal section the whole staminal leaf has a wedge-like outline, because the pollen-sacs broaden outwardly. In cross-section, as in longitudinal section, we see that the wall of the ripe pollen-sac is reduced to the epidermis and a layer of collapsed cells. The walls of these epidermal cells are provided with thickening ridges. So far as the walls of the pollen-sacs will separate from the stalk of the staminal leaf, their epidermal cells, as cross-sections teach us, show a considerable reduction in size. In order to become quite clear as to the kind of thickening of the wall of the pollen-sacs, we lift a wall from the staminal leaf with needles, and determine that they are U-shaped ridges, with which the inner and side-walls of their epidermal cells are thickened. The same thickening is present also upon the epidermal cells of the outer surface of the shields. The opening of the pollen-sacs is brought about by the wall separating from the stalk and stretching straight. The pollen-grains are ellipsoidal, studded with small knobs. Shortly before dehiscence, a small cell is cut off from the end of the

grain. In alcohol-material the contents of the pollen-grain are contracted, and unfit for examination.

The pollen-grains of *Taxus* are without vesicular appendages to the wall; these latter are not present in all Abietineæ*; and on the other hand are found, in the Taxineæ, in *Podocarpus*. In many genera more than one vegetative cell is cut off from the contents of the pollen-grain, whence arise cell-masses projecting into the interior of the pollen-grain. Amongst the Abietineæ the genus *Pinus* alone shows a single vegetative cell.

The **female flowers** of *Taxus baccata* [2] are found, like the male, in the axils of leaves of the previous year's twigs (Fig. 100, *A*); but upon other individuals, as the plant is dicœcious. The time of flowering, as we already know, is in March; in alcohol the flowers preserve very well and can be very conveniently studied after they have been laid for at least twenty-four hours in a mixture of equal parts alcohol and glycerine. The flowers apparently terminate a small shoot, but are in reality not terminal. Not infrequently two flowers are found on the same shoot (Fig. 100, at *); in rare cases we even come across monstrosities, which show a leaf-bearing shoot developing laterally from the flower (Fig. 100, *B*). First we examine the flower-axis with the lens, and determine that this begins with a lateral pair of scales, to which succeed spirally-arranged scales, gradually becoming larger. The flower itself is enclosed by three decussating pairs of scales, and only its apex shows between them. This apex shows a point-like opening, the **micropyle**. We arrange the shoot in a definite way, in order to obtain a median longitudinal section. This must pass through the middle of the pair of scales last but one under the flower. We select for the examination a somewhat older flower, already pollinized, at about the end of April, because they are more suitable for cutting, and in many respects also are more instructive. If the direction of the section has been properly observed, the structure appears as in the adjoining Fig. 100, *C*. The flower does not appear to be terminal upon the primary shoot; this on the other hand closes its development, after it has formed a secondary shoot in the axil of the uppermost scale. It is this latter which ends in the flower, after it has previously given rise to three decussating pairs of scales. Pressed on one side of the point of

* Abietineæ, a sub-order of Coniferæ, which includes the well-known genera or sub-genera *Pinus* (the Pines), *Abies* (the Firs), *Picea* (the Spruces), *Larix* (the Larches), *Cedrus* (the Cedars) [Ed.]

insertion of the secondary shoot is the growing point (*v*) of the primary shoot (to the right in the figure). Now and then, the last scale but one of the primary shoot also gives rise to a secondary shoot ending in a flower. Rarely, as we have seen (*B*), the primary shoot further develops into a leaf-bearing axis. The pairs of scales which precede the flower are to be considered as its

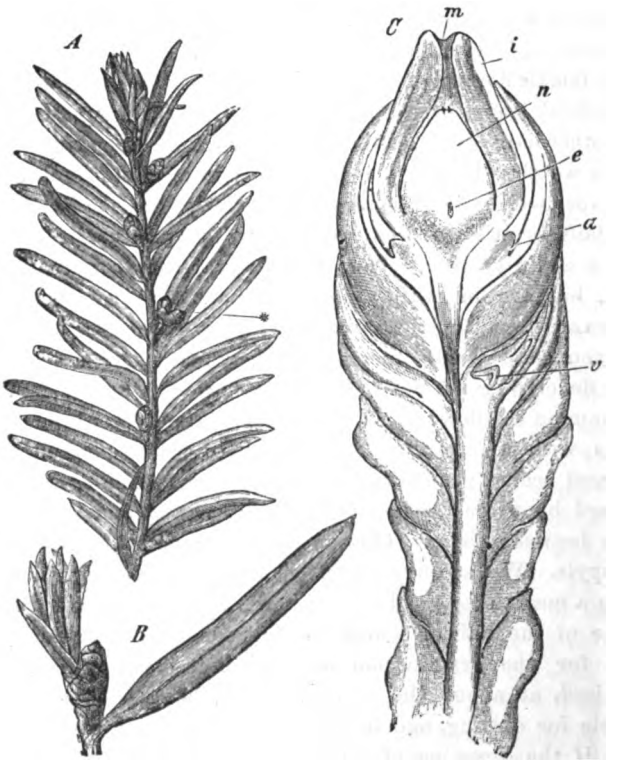


FIG. 100.—*Taxus baccata*. *A*, figure of a twig with female flowers at the time of pollination, at * two ovules upon the same primary shoot. Nat. size. *B*, a leaf with an ovule standing in its axil; the primary shoot has further developed laterally [is proliferous]. (× 2). *C*, longitudinal section through the common median plane of the primary and secondary shoot; *v*, growing point of the primary shoot; *a*, commencement of the aril; *e*, rudiment of the embryo-sac; *n*, nucellus; *i*, integument; *m*, micropyle (×18).

bracteoles; the flower itself is reduced to an **ovule**. Such is, for example, the terminal structure which we see at the apex of the secondary shoot. In the longitudinal section of this we distinguish a simple case, the **ovular integument** (*i*), which leaves above a

narrow opening, the **micropyle** (*m*) free, and in the interior the so-called nucleus of the ovule, the **nucellus** (*n*). At the base of this, only, however, in specially favourable cases, or after treatment with potash, a large cell (*e*) is to be recognised as the rudimentary **embryo-sac.**[3] As the pollen-sac resembles a microsporangium, in the same way the ovule corresponds with a **macrosporangium**; as the pollen-grains resemble microspores, so the embryo-sac a **macrospore.** Developmental researches[4] have disclosed considerable agreements between the initiation of these structures, but have at the same time shown that a progressive reduction affects the processes which amongst Phanerogamia lead to the development of the macrospore. To compare the integument with the indusium of the Vascular Cryptogams offers, however, no sufficient grounds. The integument is a newly-evolved structure on the macrosporangium of Phanerogams. Upon the stalk of the ovule of *Taxus* can be seen a small wall of tissue (*a*), which for a long time, even into June, remains stationary; later, however, begins to grow, and forms the bright-red **aril,** which in autumn surrounds the ripe seed. Upon the already pollinized flowers which we have taken for investigation, we can see the pollen-grains lying on the apex of the nucellus. Each of them has put out a short sac into the tissue of the apex of the nucellus. It is the large cell of the pollen-grain which grows out into the sac, while the small vegetative cell shrivels. The inner wall of the pollen-grain, the **intine,** forms the **pollen-tube,** while the extine, studded with small protuberances, which we have already seen upon the ripe pollen-grains, is stripped off. The pollen-grains lie in this case upon the surface of the papillose nucellar apex; while with various other Taxineæ, and their near allies, the nucellar apex is hollowed out[5] in order to receive the pollen-grains, giving rise to the so-called pollen-chamber. If we wish to know the mechanism which brings the pollen-grains to the ovule, we must make the observation in the open air, during the time of pollination.[6] If we examine the female flowers at the time when the pollen-grains are being emptied from the pollen-sacs, we shall see that each flower exudes a small drop of fluid from its micropyle. In this drop the pollen-grains, carried by the wind, are caught, and in the evening are absorbed at the same time with the drop.

The Scotch Fir (*Pinus sylvestris*) will serve as a second, and at the same time extreme, example of the structure of the female flower of the Coniferæ. The Scotch Fir is **monœcious,** so that we

find male and female flowers upon the same plant. The ovules in the Scotch Fir do not stand alone, as in the Yew, but are developed in **cones**, in which numerous ovules, inserted upon scale-like structures, are found combined. The small cones, either singly or several together, occupy the apex of twigs of the same age. They stand in the axils of bracts like those of the condensed branches, each bearing two needle-leaves, inserted lower down on the axis; their position at the end of the shoot corresponds, however, with that of the normal twig-bearing branch. The small cones are usually in the receptive state at the end of May, and, though small, are recognisable by their brown-red colour. They are stalked, and stand erect; the stalk is covered with brown scales. —Here also alcohol-material treated with glycerine can serve for the investigation. If we bring a portion removed from the axis of the cone with the scalpel under the simple microscope, and isolate it with needles, we can see (Fig. 101), that in the axils of delicate obovate bracts (*b*), somewhat fringed at their margin, arise scales (*fr*) of similar form, but fleshy, smooth-edged, provided on the inner side with a central projecting rib (*c*). These are distinguished as **fruit-scales**. At the base of the fruit-scale, right and left, is found on each side of the rib an **ovule** (*s*), with its **micropyle** turned below and towards the outer side. The edge of the **integument** is prolonged at the micropyle into two lobes (*m*), placed right and left. Bract and fruit-scale have grown together at the base, and are therefore removed together from the axis of the cone. The cones of the Abietineæ and other true cone-bearing Coniferæ are conceived to be either single flowers or inflorescences, according to the significance which is given to the fruit-scale. That is, this is either considered to be a flattened, metamorphosed axial shoot, growing in the axis of a modified leaf, which we have here called the bract, and partially adnate to the bract; or as a development of the placenta of a

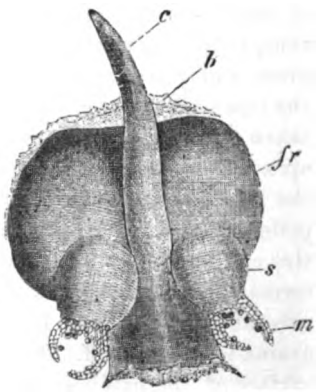


FIG. 101. — *Pinus sylvestris*. Fruit-scale *f* with its two ovules *s*, and the central rib *c*. Behind is the bract *b*. Upon the ovule the integument has grown out into two prolongations, *m* (× 7).

carpellary leaf, which we have hitherto called the bract. In the former case, therefore, we should have a **bi-ovular branch** in the axil of each **bract**, in the second a **bi-ovular placenta** on the upper side of a **carpellary leaf**. In the former case, the cone would therefore be an **inflorescence** composed of many fertile axillary branches; in the second, the cone would be a **single flower** composed of numerous open carpellary leaves.—The remarkable structure of the fruit-scale is explained by the machinery for **pollination**,[7] which can only be followed upon fresh material at the time of pollination. As soon as the male flowers begin to free their pollen, we can demonstrate an elongation of the axis of the cone, whereby the fruit-scales, together with the bracts appertaining to them, are separated. The pollen can now fall upon the lifted fruit-scales, slip down them, and, guided by the projecting rib, come between the two prolongations of the integument. Later on, these prolongations curve inwards, and in this way carry the pollen into the micropyle, and to the apex of the nucellus. After full pollination the fruit-scales soon close together again by their edges, and are glued together by resin. The bracts do not further develop, nor does the central rib of the fruit-scale, which is of no further use. The red colour of the cone passes over into brown, and then into green; when ripe, again becoming brown; and the cone slowly sinks, and finally takes a pendant position.

We will now turn our attention to the further changes which take place in the pollinated ovules of the Coniferæ.[8] With the structure of the ovule we have already become acquainted in *Taxus*, and have proved that at the time of pollination only the first rudiments of the embryo-sac were present. After this a further development of the ovule takes place, always variously quickly, according to the greater or less time which has to separate the periods of **pollination** and of **fertilization**. In *Taxus*, fertilization takes place about the middle of June in the same year; in the Scotch Fir not until the next year, about thirteen months after pollination. In the Spruce Fir (*Abies*, *Picea*), pollination and fertilization are separated by about six weeks only. We will consequently, in what follows, keep to the Spruce Fir, because this offers many advantages for the investigation. It would lead us too far to follow step by step the enlargement of the **embryo-sac**, the origin of the tissue of the **prothallus** (**endosperm**) and of the sexual organs in its interior, the increase in

size and corresponding differentiation of the entire rudimentary seed. We will therefore turn at once to the stage in which the oospheres are fully formed and in a receptive condition. This condition, in the common Red Fir (*Picea vulgaris*, Lk.), is reached about the middle of June, the fertilization is then completed in the course of a few days. Either fresh or alcohol material must be at command. For this investigation, alcohol material is better suited than fresh, as it shows the oosphere fixed. It is, above all, recommended not to lay entire cones, but separate fruit-scales, in the alcohol. Before cutting the alcohol material, it should be transferred, as we have already repeatedly done, to a mixture of equal parts alcohol and glycerine for at least twenty-four hours.—In beginning the investigation, we first inform ourselves as to the appearance of the entire scale. This is obovate, shows below, on its inner surface, the two rudimentary seeds, also already the outlines of the **wings**, which later on will be separated, with the ripe seed, from the inner surface of the fruit-scale. On the outer surface of the fruit-scale, and below, can still be found the bract now appearing comparatively very small. The ovule to be cut can be easily separated uninjured from the fruit-scale with the points of the needles. We prepare longitudinal sections of it between thumb and forefinger. Cutting is made more difficult by the integument having become comparatively hard, therefore we must somewhat modify our method of preparation. We cut the ovule in two with the scissors at about half its height; we then take the upper half of the ovule, *i.e.*, that which contains the apex of the ovule, between the fingers, and with the forceps withdraw out of the cut surface the upper part of the embryo-sac, together with the nucellus. Through these soft parts longitudinal sections can now be readily made. Staining reagents are only to be used with great precaution, as they stain the entire protoplasm of the oospheres, and can easily make them opaque. We first examine the longitudinal section of a receptive ovule with a low power. The entire ovule, with integument, is cut perpendicularly to its surface of insertion; it is displayed, therefore, in median longitudinal view (Fig. 102). We see in it the **integument** (*i*), which develops into the skin of the seed, and from half its height is separated from the nucellus; the **nucellus**, bearing upon its apex **pollen-grains**, which partly are external, and partly lie sunk in its tissue; or may even show **pollen-tubes** (*t*), developed from these pollen-grains, which

pierce the upper part of the nucellus, in order to reach the external layer of the embryo-sac; the **embryo-sac** (*e*), of elliptic outline, filled with **endosperm** (or, more correctly, prothalloid tissue); the **archegonia**, here known earlier as **corpuscula**, whose ventral portion (*a*) is easy, but neck more difficult to recognise; in the interior of each archegonium is an **oosphere** (*o*), which in alcohol material is noticeable from its yellow-brown colour, and shows a central large **nucleus** (*n*); and lastly, under the ovule, the commencement of the **wing** (*s*). If we prepare a similarly-directed section through a fresh ovule of the same age, we shall again find the same relations; but very commonly the contents of the archegonium will have run out. If the section has laid bare individual archegonia, without opening them, the oospheres will appear as yellowish frothy masses of protoplasm, in which the central nucleus is scarcely distinguishable, or else, in the best cases, has only the appearance of a large central vacuole. The oospheres quickly suffer under the influence of the water taken from the neighbourhood; if the section is to be kept for a longer time, it is recommended to use as fluid for observation white of egg diluted with water, to which, for greater durability, a little camphor has been added.[9] In such preparations **the neck** of the archegonium is not difficult to see (Fig. 102A, *A c*). It consists of from two to four stages of cells. Under the neck is to be

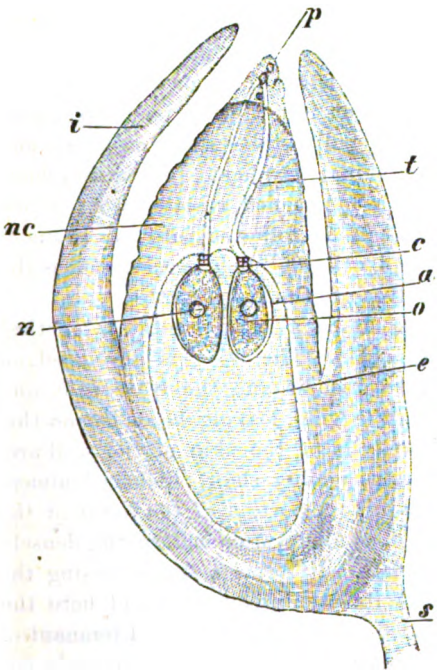


FIG. 102.—Median longitudinal section through a receptive ovule of *Picea vulgaris*, Lk. *e*, Embryo-sac filled with endosperm; *a*, ventral portion, and *c*, neck of an archegonium; *n*, the nucleus of the oosphere; *nc*, the nucellus of the ovule; *p*, pollen-grains upon and in the nucellar apex; *t*, pollen-tubes, traversing the nucellus; *i*, integument; *s*, the wing of the seed (× 9).

found a small cell (*cl*), which corresponds with the **ventral canal-cell** of the Vascular Cryptogams; the oosphere divides, in order to form it, shortly before it is ripe. The ventral part, or body, of the archegonium is surrounded by a layer of flattened cells, richer in cell-contents, like to the layer which we saw around the body of the archegonium in Ferns.—In order to inform ourselves as to the number and position of the archegonia, we prepare a number of successive cross-sections through the upper part of the ovule. In this way we show that from three to five archegonia, arranged in a circle, stand in the apex of the embryo-sac. Sections which have laid bare the apex of the embryo-sac show us the neck of the archegonia in apical view as rosettes of six or eight cells. If our material has been gathered at the time of fertilization, we may be able to follow individual pollen-tubes to an oosphere, and find in the lower end of individual oospheres [or, as they have now been fertilized, **oospores**] a four-celled rosette, from which four connected sacs, or tubes, can be followed into the tissue of the prothallus. The four end cells of such sacs produce the **embryo**, the long sacs themselves are the **suspensors.**[a]

The seed ripens in October. It then easily separates, together with the wing, from the fruit-scale. The wing is developed on the inner side of the seed, between it and the fruit-scale, and the seed later falls easily from the wing, leaving behind upon this a corresponding hollow. The cells of the skin of the seed are, as cross and longitudinal sections readily show, thickened almost to the obliteration of their cavity. A portion of the tissue of the prothallus remains in the seed, as **albumen** or **endosperm**, densely filled with **reserve food materials**. It forms a sac, enclosing the embryo. This sac is open at its micropylar end, and here the **radicle** of the embryo is placed against the displaced remnant of the nucellus. The embryo can be easily made out in seeds cut in the direction of their length. It looks like a cylinder, gradually getting thicker towards the cotyledonary end. In consequence of being filled with reserve food-materials it is white, and as opaque as the albumen or endosperm of the seed. We prepare a median longitudinal section through the seed between the fingers, and lay it in carbolic acid diluted with alcohol. The figure becomes very beautifully clear, far better than in potash, and better even than in chloral hydrate, so that we can follow every row of cells. We see (Fig. 103) that the **cotyledons** (*c*) do not reach quite a third of the whole length of the embryo. At

[a] See note *a* on page 310*a*.

the base between them is to be seen the growing point [*punctum vegetationis*] of the embryonic stem [the **plumule**]. The stem (**caulicle**) itself, which is distinguished as the hypocotyledonary axis, or **hypocotyl** (*h*), passes without clear limitation into the root (the **radicle**). This is for the most part represented only by a growing apex, which shows clearly in the interior of the body of the embryo, but is in reality only the apex of the **plerome** (*pl*) of the root, while the cell-rows of the **cortex** [**periblem**] of the hypocotyl pass directly into the parabolic layers of the **rootcap** (*cp*), a relation which recurs in all roots of the Gymnosperms, inasmuch as we can see the cell-rows of the cortex of the body of the root pass over direct into the cell-layers of the root-cap (cf. *Thuja*, p. 186). The root-cap is traversed in the direction of its long axis by a distinctly-marked column of tabular cells, arranged in straight rows. In the hypocotyl the tissue of the **pith** (*m*) already begins to show, and around this the elongated cells of the **procambium ring** (*op*), in which the fibro-vasal bundles will make their appearance. These cells can be traced, moreover, for a short distance along the median section of the **cotyledons** (compare the Fig.). Thus in the embryo the essential parts of the future plant are already established.

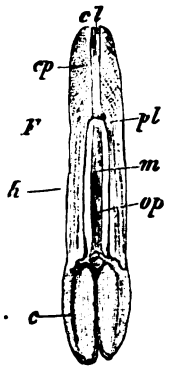


FIG. 103.—Longitudinal section through the ripe embryo. *c*, cotyledons; *h*, hypocotyl; *pl*, growing apex of the plerome; *cp*, root-cap; *cl*, its central column; *m*, pith; *op*, procambium ring in the hypocotyl (× 10).

NOTES TO CHAPTER XXVII.

[1] Upon this compare: Strasburger, *Coniferen und Gnetaceen*, p. 120. Eichler, *Blüthendiagramme*, Bd. I., p. 58. Goebel, *Grundzüge*, p. 363.

[2] Strasburger, *l.c.*, p. 2.

[3] Strasburger, *Angiospermen und Gymnospermen*, p. 109.

[4] Strasburger, *l.c.*, p. 109. Goebel, *Botanische Zeitung*, 1831, Sp. 681.

[5] Strasburger, *Jenaische Zeitschr. f. Naturw.*, Bd. VI., 1871, p. 250.

[6] The same, p. 250; *Conif. u. Gnet.*, p. 265.

[7] Strasburger, *Jen. Zeitschr.*, Bd. VI., p. 251; *Conif. u. Gnet.*, p. 267.

[8] Compare Strasburger, *Befr. b. d. Conif.*; *Coniferen und Gnetaceen*, p. 274. *Befruchtung und Zelltheilung. Angiospermen und Gymnospermen*, p. 140. Goroschankin: *On the Corpuscula and fertilization in Gymnosperms*, in Russian, 1880.

[9] Strasburger, *Befr. b. d. Coniferen*, p. 8.

[N.B.—*Picea vulgaris*, Link, of the text, is *Pinus Picea*, and *Abies Picea* of various authors.]

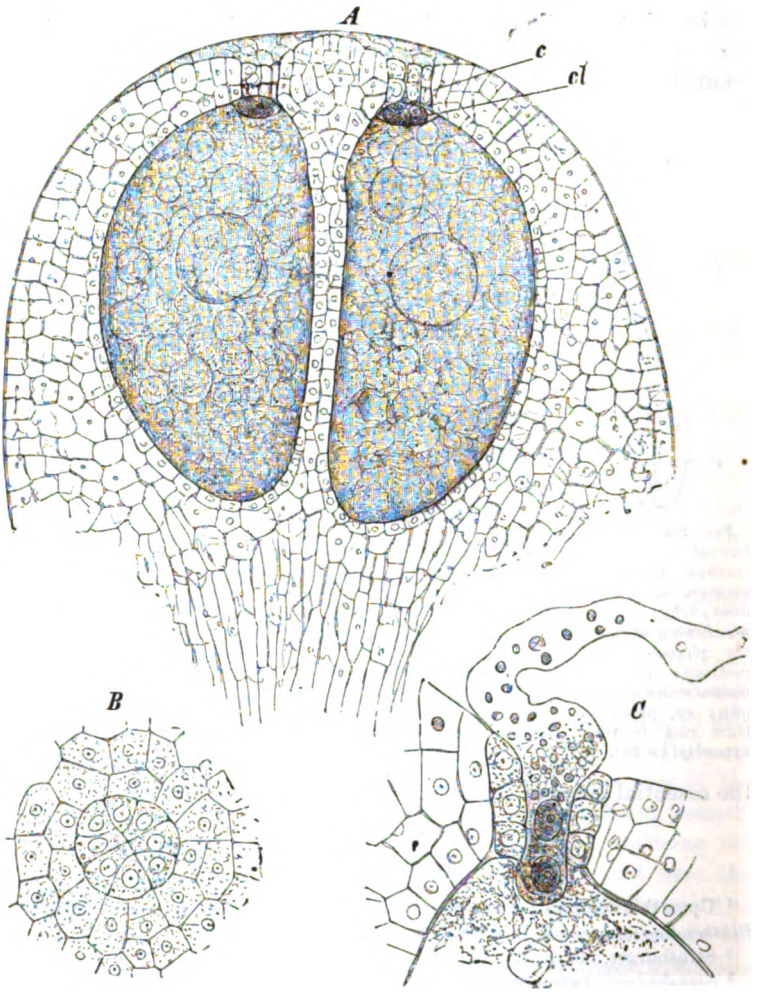


FIG. 102A.—*Picea vulgaris*, Lk. From fresh material. *A*, a longitudinal section through the apex of the embryo-sac, with two archegonia; *c*, neck of the archegonium; *cl*, ventral canal-cell. *B*, apical view of an archegonium. *C*, penetration of a pollen-tube through the canal (*A* × 100; *B* and *C* × 250).

[*Note* * *to page* 309.]

Some further details of the embryology of *Pinus* may be here given. Fig. 102A, *B*, shows the neck of the archegonium, seen in cross-section. If fertilization has been accomplished, we may perhaps be able to trace individual pollen-tubes right to the embryo-sac, or may even be able to follow it into the archegonium

(Fig. 102A, *C*). They penetrate between the cells of the neck, and reach the oosphere (*C*). This may take place under the influence of some substance given off from the oosphere, and which acts as a chemical stimulus upon the pollen-tube. The pollen-tubes arrive at the embryo-sac through the conducting tissue of the nucellus, growing in the direction in which they are best nourished. The pollen-tube is densely filled with fine granules, which the addition of iodine proves to be starch. In specially favourable cases we can see near the apex two nuclei, surrounded by protoplasm and behind which are the masses of starch. These nuclei are much clearer in alcohol material, from which *C* in Fig. 102A, was obtained.—The subsequent phenomena must be studied in alcohol material. According to the approved method, we prepare numerous delicate sections, which we examine in glycerine. Sections which are too thick can be rendered more transparent in potash, but this reagent must be used with great care; the hardened oospheres can also be removed from the archegonia by needles, and examined by themselves. In oospheres as yet unfertilized (Fig. 102B, *A*), we see the approximately central nucleus (*on*), which on the side towards the neck of the archegonium always appears denser. We can also often see the ventral canal cell (*cl*); a nucleus in its interior is usually no longer identifiable, as it is early disorganized. If the pollen-tube has penetrated to the oosphere, we may be able to see a nucleus in the oosphere, under its tip (Fig. 102B, *B sn*), which is inferior to the oosphere-nucleus in size. In order to obtain such a preparation much patience is no doubt often necessary. The small nucleus has come from the pollen-tube, and is distinguished as the **spermo-nucleus**, that appertaining to the oosphere being, on the other hand, known as the **oo-nucleus**. The tip of the pollen-tube of *Picea* is finely porous, in species of *Pinus* it shows a clear pit, but this would not serve for the passage of the nucleus. The membrane of the apex of the pollen-tube is, however, very soft, and would offer no considerable resistance to the passage of the nucleus, just as the swollen wall of the apex of the oogonium of *Vaucheria*, offers little resistance to the passage of the mass of plasma which is squeezed through it prior to fertilization (*vide* p. 253). Only one nucleus of the pollen-tube fertilizes the oosphere; while the other, as well as the starch-grains, is dissolved, and may serve towards the nourishment of the oospore. The spermo-nucleus (*sn*) which we see in the oosphere is equivalent to a spermatozoid, and differs

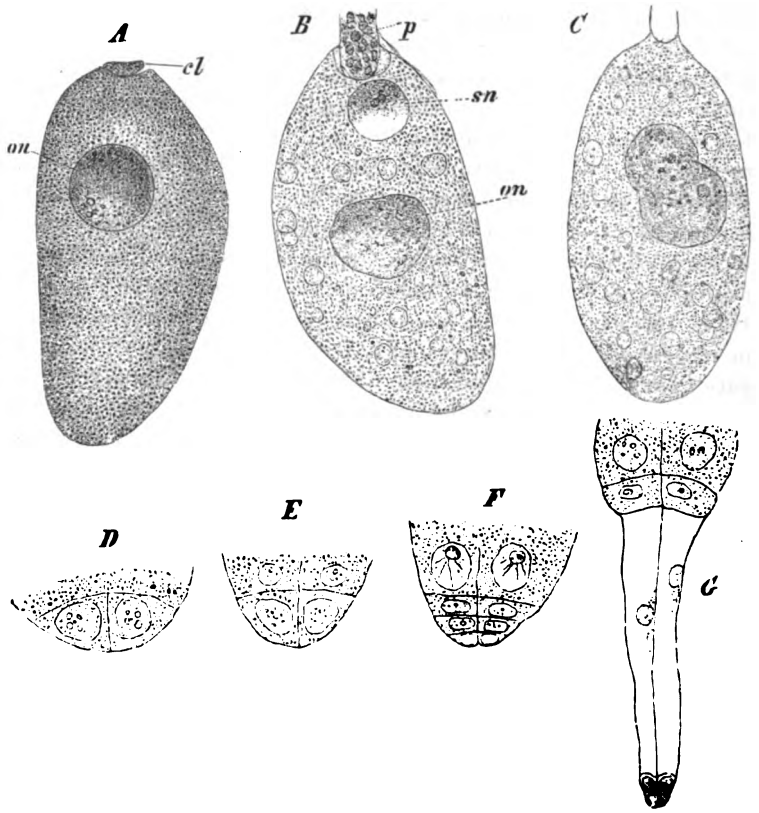


FIG. 102B.—*Picea vulgaris*, Lk. *A*, a ripe oosphere with nucleus, *on*; and ventral canal cell, *cl*. *B*, an oosphere during fertilization; *sn*, the spermo-nucleus; *on*, the oo-nucleus; *p*, the tip of the pollen-tube. *C*, an oosphere during fertilization, showing the conjugation of spermo- and oo-nucleus. *D*, the four nuclei in the end of the oospore remote from the neck, only two of which can be seen, since they all lie in the same plane. *E*, the nuclei have divided; four nuclei lie now at the end, and four others more in the body of the oospore. *F*, three stages of cells are formed in the end of the oospore. *G*, the middle stage has elongated, and carried the lowest stage into the endosperm. The cells of this lowest stage have divided. From alcohol material (×90).

from the spermatozoid of the Vascular Cryptogams (which, as their development shows, consist of little besides nuclear substance), in the main only in its simple nuclear form, and the want of organs of motion. In these cases, where the spermo-nucleus is taken by the pollen-tube to the place where it is wanted, these latter have become superfluous, and the simplification of its form is no doubt due to the same causes, since the corkscrew shape of the spermatozoids

of Vascular Cryptogams certainly has reference to their movement. The spermo-nucleus thus penetrated into the oosphere soon increases in size, just as do the spermo-nucleus-forming spermatozoids of animals after their entrance into the ovum, and moves towards the oo-nucleus. Preparations may be found which show the two nuclei in course of fusion (*C*). The nucleus resulting from their fusion is distinguished as the **embryo-nucleus**. The next stages take the embryo-nucleus into the end of the oospore remote from the neck, where, by repeated bipartition, it forms four nuclei lying in the same plane (*D*). These are separated from one another laterally by partition walls. They repeat their bipartition towards the interior of the oospore, and become separated from one another in this direction also (*E*). The four nuclei lying at the end of the oospore again divide in the same direction, and the resulting nuclei which lie nearest to the end of the oospore once more divide. Ultimately therefore, at that end of the oospore which is farthest from the neck, we find three stages, each of four cells, and above these, in the general body of the oospore, four free nuclei (*F*). These free nuclei enlarge very considerably, and later on sink. Of the three stages of cells, that which is nearest to the neck remains as a four-celled rosette at the base of the archegonium, the median ones elongate, forming the "embryonal tubes," from which the **suspensor** is formed, and carry the cells which were most remote from the neck of the archegonium down into the tissue of the prothallium (*G*). These last cells constitute the rudimentary embryo. They are distinguished from the first by their richer contents, and soon divide into two (so early as *G*), and then into three stages.

We can use the same fir, *Picea vulgaris*, Lk., in order to study older ovules with rudimentary embryos. We can either use the material fresh, and at intervals of about eight days, or the material can at similar intervals be laid in alcohol. Such material can only come into consideration provided the investigation is not spread over a long period.—The rudimentary embryo rapidly increases in size and in number of cells by the formation of periclinal, anticlinal, and radial walls, and takes the form of the adjoining Fig. 102c, *A*. These divisions preclude from the very first the existence of an apical cell. After the embryo has further enlarged, its hinder end begins to develop tubularly, and adds to the embryonal-tubes, so that the "suspensor" formed from these becomes more and more massive. The rudimentary embryo itself

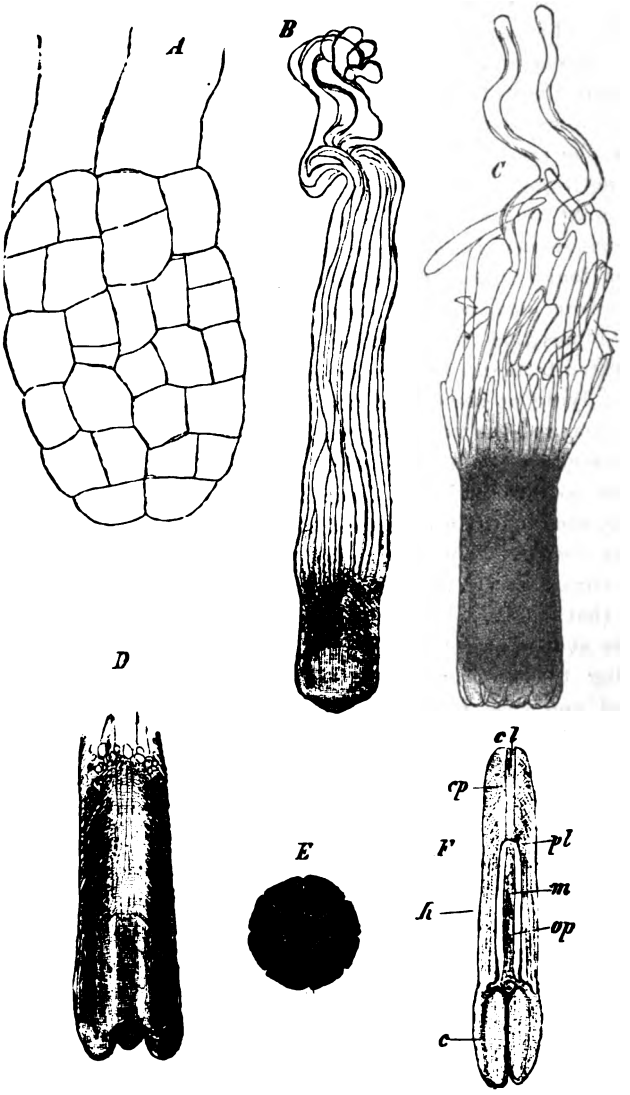


FIG. 102c.—*Picea vulgaris*. *A*, young rudimentary embryo in optical section (×240). *B*, older rudimentary embryo in optical section. The rudimentary root and growing point of the stem are already complete (×27). *C*, half-ripe embryo seen from the outside. *D*, the same in longitudinal section; and *E*, in apical view (×27). *F*, longitudinal section through a ripe seed, showing: *c*, cotyledons; *h*, hypocotyl; *pl*, apex of the plerome of the root; *cp*, root-cap; *cl*, its central column; *m*, pith; *op*, procambial ring in the hypocotyl (× 10).

assumes a cylindrical form, becomes opaque, and is then very sharply contrasted with the transparent suspensor. When the opaque portion has obtained a length of about 0·5 mm. the rudiment of a root can (after the use of potash, creosote, or chloral hydrate has made it transparent) be made out in its interior. This is differentiated at about 0·15 mm. distance from the apex, and always by periclinal divisions within a layer of semi-globularly arranged cells (Fig. 102c, *B*). Henceforth this root-apex adds to the length of the embryonic axis posteriorly by its growth. Soon the other end of the embryonic axis bulges in its middle portion (*B*), in order to form the growing-apex of the stem. Around this then arise in considerable number the rudiments of the seed-leaves or **cotyledons** (*C*, *D*, and *E*). Now all the parts of the embryo are present, and need only to grow in order to attain the structure visible in the ripe seed.—Hitherto we have concerned ourselves solely with the more strongly developing embryo, which ultimately is alone present; but in reality several if not all of the archegonia give rise to rudimentary embryos. All these rudiments grow back into the long axis of the body of the prothallium; that one, however, which originates before the others, and first, therefore, makes use of the food-materials stored in the tissue of the prothallus, develops more strongly, and ultimately crushes back all others. At the time when the cotyledons show themselves, the rudimentary embryo already lies with its apex at the base of the embryo sac. With further growth the radicular end must now be again thrust back, and ultimately attains the position whence the whole development started. The suspensor is pressed upwards, and ultimately reduced to a knot of disordered cells. The individual cell-rows constituting it separate easily from one another (*C*).

CHAPTER XXVIII.

THE ANDRŒCIUM OF ANGIOSPERMS.

Material Wanted.

Flower-buds of various ages of the Day Lily (*Hemerocallis fulva*). Fresh, or in alcohol. July. Any other large liliaceous flower will do—*e.g.*, any Lily, Tulip, Hyacinth—thus providing fresh material for various times in the year.

The same of *Tradescantia virginica* (July), or of a *Leucojum*.

Flower-buds, ready to open, of the Evening Primrose (*Œnothera biennis*), *Epilobium*, or *Fuchsia*. Fresh.

Flowers of Hollyhock or Mallow (July), any Curcurbit, *Calluna vulgaris* (the Ling), or other heath, *Azalea*, or *Rhododendron*. Fresh, or in alcohol.

Quite freshly-opened flowers of Sweet Pea, Pæony, or Everlasting Pea. Fresh.

The male sexual organs of an **Angiospermous flower** form collectively the **androecium**. The individual **stamen**[1] consists of a usually thread-like stalk, the **filament**, and the **anther**. This last is formed of two longitudinal halves or **anther-lobes**, which are separated by the upper part of the filament, the so-called **connective**. It is desirable, however, to include the connective with the anther. In the tissue of each anther-lobe are usually immersed two compartments, or **pollen-sacs**. Each compartment corresponds with a **microsporangium**.—We first inform ourselves about the stamen of some one of the large-flowered Liliaceæ; for example, *Hemerocallis fulva*, a hardy herbaceous perennial very widely cultivated in gardens, or any of the still more universally cultivated white or tiger Lilies, Tulip, Crown Imperial, etc., will do equally well. The yellow filament is here very long, becomes thinner towards its upper end, and tapers very sharply at the place of insertion of the anther. This latter is brown in *Hemerocallis*, and movable (versatile) upon the filament. The connective can be followed along the outer side of the anther as a

thin stripe between the two anther-lobes. The ripe **pollen**, observed dry upon the object-slide, shows the form of coffee-berries the general form in Liliaceæ. It appears yellow, ornamented with a network of ridges on its surface. If, while examining, we allow water to enter from the edge of the cover-glass, we see that each pollen-grain, as soon as wetted, levels up its furrow, strongly bulges out on the corresponding side, and takes the form of a unilaterally flattened ellipsoid. The membrane of the previously furrowed part shows a relatively considerable thickness, is colourless, has no markings, and is limited sharply against the sculptured, brownish membrane. Careful focussing of a pollen-grain in a favourable position shows us that only a single skin surrounds the pollen-grain, that the colourless part thins off at its edges and passes direct into the coloured. Between the grains in the preparation orange-red oil is everywhere present, and clings also to the surface of the grains, giving to them in the dry state their yellow coloration. The contents of the pollen-grain appear grey and finely granular. After a short time, during which the pollen-grain slowly and continuously enlarges, it bursts and empties its contents, in the form of a worm, into the surrounding water. In sugar solution of suitable concentration the grains round off without bursting, and can be examined uninjured. If we allow concentrated sulphuric acid to act upon the pollen-grains, the colourless smooth part of their wall is at once dissolved, while the sculptured, brownish part, on the other hand, resists: it is cuticularized. The cuticularized portion has therefore, in the open anther, where the pollen-grain is furrowed, to serve for the protection of the entire grain. As can be seen upon the dry grains, the edges of the cuticularized parts are in contact along the fold, or furrow, so that the non-cuticularized portion lies completely concealed in the fold. It first comes into view when the grain swells, and grows out into the pollen-tube. An **extine** and an **intine**—*i.e.*, a special outer and inner coat—is, however, as we see, not to be distinguished upon the pollen-grains of *Hemerocallis*, because the wall nowhere shows a double composition. Its cuticularized portion functionates as an extine, while the non-cuticularized part behaves just as does the intine in other cases.—Under the influence of sulphuric acid the structure of the cuticularized membrane is very clear. Examined from above with strong magnification, it shows a meandering network with elegantly wavy walls. In many meshes we can see lying a blue body, with

irregular outline, which represents the oil, previously yellow, but become blue with sulphuric acid. The cuticularized membrane itself has become yellow. If we now focus for the optical section, we recognise readily a connected basal wall, upon which are the projecting ridges. The ridges are swollen at their outer angles, so that in optical section they appear club-shaped. In surface-view the areas at the bottom of the meshes appear finely dotted, and the optical section shows that these dots are in reality minute knobs, which are upon the basal wall. After some hours' action of the sulphuric acid, the membrane assumes a red-brown coloration, while the contents of the pollen-grain, which have come out, are at the same time stained rose-red, a reaction which protoplasm often shows with sulphuric acid.[2]

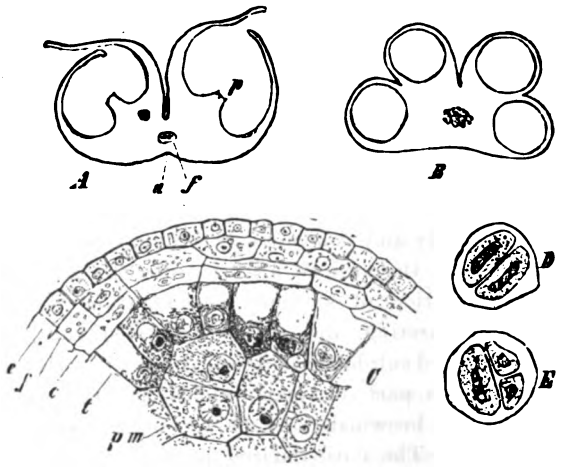


FIG. 104.—*Hemerocallis fulva*. *A*, cross-section through an almost ripe anther, with pollen-sacs opened by cutting; *p*, the partition wall between the sacs; *f*, fibro-vasal bundle of the connective; *a*, groove along the connective (× 14). *B*, cross-section through a young anther (× 28). *C*, Part of the previous cross-section of a sac. *e*, epidermis; *f*, the fibrous layer [mesothecium] formed later; *c*, the layer to be displaced; *t*, the tapetal layer, to be resorbed later on; *pm*, pollen mother-cells (× 210). *D* and *E*, division of the pollen mother-cells (× 240).

We now prepare cross-sections through the anthers; first it would be well to turn to a flower-bud only about two-thirds grown, and cut cross-sections through this. The sections of the perianth are then removed from the preparation with the needles. Although we have chosen so young a flower for investigation, we nevertheless find all the pollen-sacs open. Their opening is effected very easily, and is brought about by the pressure of the razor in cutting. The adjoining figure (Fig. 104, *A*) will assist our conception. The walls of the pollen-sacs separate away (at *p*) from the partition walls separating the two sacs of each anther-lobe.

Hence such an anther when ripe is commonly, by systematists, said to be "2-celled." They thus reduce their curvature. The two anther-lobes are connected together by the narrow connective, traversed by a **fibro-vasal bundle** (*f*). If we now examine the cross-section with stronger magnification, we see most outwardly the **epidermis** of flat cells filled with violet cell-sap. These epidermal cells are bulged outwards. At the edges of the walls of the sacs they are rapidly reduced to a small height. Here the separation from the middle partition wall takes place. **Stomata** are scattered over the whole surface of the anthers. A small **air-chamber** lies under each of these. To the epidermis follows, on the wall of the sac, a single layer of comparatively high cells, with annular thickenings, the so-called **fibrous-layer** or **mesothecium**. The rings on these cells are arranged perpendicularly to the surface; they pass over partially into spiral thickenings, and branch frequently into a network. Towards the dorsal side of the anther the walls of the sac become gradually thicker, the fibrous layer being doubled. The remainder of the body of the anther is likewise constructed of fibrous cells. Only the cells which surround the fibro-vasal bundle of the connective, and those (*p*) which form the partition wall between the pollen-sacs, are without thickening ridges. In order to prepare surface-sections of the anther, we again select a flower-bud about two-thirds developed. The surface-sections show that over the sacs the epidermal cells are longitudinally, the cells of the fibrous layer, on the other hand, are transversely, elongated. Not so on the dorsal surface of the anther, where the fibrous cells appear more isodiametric. Over the sacs the thickening ridges on the outer side of the fibrous cells are weaker, often scarcely recognisable. In drying, the cells of the fibrous layer contract in tangential direction transverse to the long axis of the loculus; they are hindered in radial contraction by the thickening ridges. On the outer surfaces, where the thickening ridges are weak, the contraction of the fibrous cells has more effect, whence the outward curvature which results in the rupture of the loculi.[3]—Often in Angiosperms, as in *Taxus*, the thickening is entirely wanting on the outer surface of the fibrous cells of the wall of the sac, so that the thickening ridges show U-shaped or basket-shaped figures open towards the exterior; it is clear that such a disposition assists the wall of the sac in becoming concave on its outer side.—In order to study closely the relations of the filament with the anther, we prepare a median longitudinal section

which falls between the two anther-lobes, through the upper part of the stamen. We see the filament thin off very strongly at the point of insertion of the anther. Its bundle enters into the connective, and passes through it, gradually becoming attenuated, almost to the apex of the anther. The non-fibrous cells, surrounding the fibro-vasal bundle, which we saw in the cross-section, can likewise be followed out of the filament into the connective. In order to obtain closed pollen-sacs in cross-sections, we must go back successively to younger and younger flower-buds so long as it proves necessary (Fig. 104, *B*).

Now prepare cross-sections through a flower-bud about ¼ inch high, and we shall find the walls of the sacs consisting, besides the epidermis (Fig. 104, *C*, *e*), of two or three layers of flat (*f*, *c*), and one layer of radially elongated cells (*t*).[4] These last surround the entire sac. The interior is filled with polygonal **pollen mother-cells**.

If we next prepare cross-sections through a flower-bud about ½ inch in height, we shall see the pollen mother-cells already isolated and in course of division. These pollen mother-cells are recognisable by their white, thick, strongly refractive wall; their contents are divided into two, or already into four cells, which lie in one (Fig. 104, *D*), or in two planes at right angles (Fig. 104, *E*). These **pollen-grains**, therefore, like spores, are produced by quadripartition inside their mother-cells. The wall of the anther is lined by **tapetal cells**, which are filled with yellow-brown contents. These constitute the innermost layer (*t*) clothing the sac. In the next older flower-buds the walls of the pollen mother-cells are dissolved; the young pollen-grains lie free; the tapetal cells have for the most part lost their independence, their contents have penetrated between the young pollen-grains. The layer of flattened cells (*f*) underlying the epidermis has strongly developed, and forms the fibrous layer, while the next inner layer is crushed and disorganized. Ultimately, as still older buds show, the unconsumed portion of the tapetal cells, especially in the periphery of the sac, takes on an intense yellow-brown coloration, a glistening oily appearance, and so forms the oily substance which clings around and upon the pollen-grains.

The species of the genus *Lilium* agree with *Hemerocallis*. The processes of differentiation in the anther commence here, however, later. In flower-buds of the white Lily, *Lilium candidum*, of *L. croceum* and others, four-fifths of an inch high, the pollen mother-

cells first begin to divide. In cross-sections through fresh flower-buds the large tapetal cells are very striking from the yellow-brown coloration of their contents. The hypodermal cells, as well as all the others which are later on provided with thickening ridges, are densely filled with starch-grains.

Funkia ovata [May] provides likewise a very favourable object for investigation, and agrees with *Hemerocallis* and *Lilium*, as also do *Agapanthus umbellatus* [Greenhouse, April] and many others. *Tulipa* [the Tulips, April, May], and *Hyacinthus orientalis* [the Hyacinth, January—May] are likewise good to use. In *Tulipa* the filament under the anther tapers so sharply that this latter will draw off; in *Hyacinthus* the anthers are almost sessile on the perianth segments.

Tradescantia virginica does not cut so well, but we examine them with respect to their pollen-grains. Cross-sections through flower-buds which have attained about two-thirds of their definite length, show us the two halves of the anther separated by a connective elongated somewhat considerably in cross-direction. The walls of the sacs are already reduced to two layers, and the thickening ridges already formed in the inner layer. The young pollen-grains lie embedded in a yellow-brown substance, the origin of which from the tapetal cells is already known to us. The partition wall between the two sacs of each anther-lobe is here strongly developed, and projects so far, that externally scarcely any depression between the two sacs is to be seen. At the place of insertion of the walls of the sac upon the partition-wall, the fibrous layer ends suddenly, and here also the dehiscence takes place later on. Surface examination of the walls of the sacs shows in this case also a longitudinal direction of the epidermal cells, a radial direction of the fibrous cells, and an almost complete absence of thickening ridges on the outer wall of the cells.

If we examine with a lens the stamens of a bud which is ready to expand, we shall see the beautiful sulphur-yellow anthers fixed upon violet filaments covered with violet hairs. The dry pollen-grains are folded (or grooved) on one side (Fig. 105, *A*).

In water the fold is levelled out, and the grains become almost ellipsoid; but the side which was furrowed is more strongly bulged. Its membrane is decorated with fine sinuous lines; the furrowed side also shows this structure, and is distinguished only by its somewhat brighter coloration, and somewhat weaker cuticularization. In the finely granular contents can be distinguished two

brighter homogeneous-looking spots (*B*). These are the two nuclei, of which the one appears worm-shaped, and the other elliptic. The other contents of the pollen-grains are pretty uniformly finely granular. The pollen-grains after some time begin to flatten, whereby the nuclei, together with the contents, are pressed out. The **two nuclei** can be seen very beautifully if the pollen-grains are crushed in a drop of acetic methyl-green, or acetic iodine-green. The worm-shaped nucleus stains more deeply, and in coming out often elongates considerably. If the pollen-grains are placed in the reagents in question, but without crushing, the nuclei show in their natural position inside the grain, the worm-shaped nucleus always staining very strongly, the elliptic, on the other hand, somewhat more weakly. The rest of the pollen-grain remains at the same time unstained. If the pollen-grains in water have a drop of potassium-iodide-iodine solution added, we see, after crushing the grain, numerous small blue starch-granules in the extruded yellow-brown contents.—If we go back to the younger flowers, and remove the anthers from a bud about $\frac{1}{4}$ inch long, and crush them in water, we shall see part of the pollen-grains with one nucleus, part, as in Fig. 105 *C*, where two nuclei lie close together. These two nuclei are, however, separated by a curved partition wall, which encloses one nucleus together with a little protoplasm. This cell, in basal outline almost circular, lies always upon the flatter side of the grain, which later on is opposed to the fold, or furrow. In somewhat older flower-buds we can see that this cell has separated from the wall of the pollen-grain, and lies free in the contents of the grain. It has elongated, and correspondingly thinned, and at the same time tapered at both ends; with the exception of the two ends, it is filled by its nucleus.[5] In pollen-grains which are almost ripe, the special boundary around this nucleus has disappeared; it lies therefore completely free, and has elongated still more vermiformly. Comparison with the Gymnosperms induces us at first to

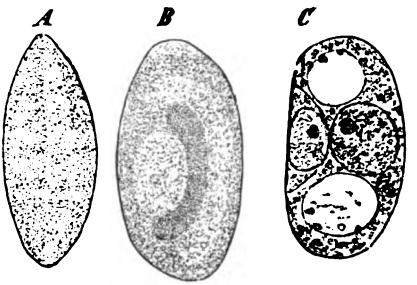


FIG. 105.—*Tradescantia Virginica*. *A*, pollen grain dry; *B*, in water; *C*, young pollen-grain in water, showing the reproductive and vegetative cells (× 540).

consider the small cell as the vegetative one; in point of fact, however, it is the **generative** cell, and it is its more deeply staining nucleus which effects fertilization.[6]—These observations can, as far as the youngest stages are concerned, be carried on in pure water; for the oldest stages we must bring acetic methyl-green or acetic iodine-green to our aid.—The species of *Leucojum* [Snowflake] agree with *Tradescantia*.

The Crown Imperial, *Fritillaria imperialis*, presents another favourable object for investigation. In sections of alcohol material, first placed in water, and then stained with logwood, the two nuclei in each pollen-grain can be seen very beautifully.

If we open a bud of *Œnothera biennis* [the Evening Primrose] which is ready to expand, we shall find that the anthers have already dehisced and set free their pollen. These latter are suspended between the anthers by cobweb-like threads. If such threads are stretched out upon an object-slide, they appear under the microscope as exceedingly delicate threads, partly stretched straight, partly tangled. The pollen-grains in a dry state are opaque, but their three-cornered form is at once noticeable. In water, with stronger magnification, they show as flattened, symmetrically triangular bodies, with rounded projecting angles. At the base of each of these rounded corners an annular thickening of the membrane of the pollen-grain is to be seen. The contents of the pollen-grain appear finely granular; the two nuclei are only recognisable in the contents of the ripe grain with extreme difficulty. In sulphuric acid the pollen membrane assumes a red-brown colour. By it an outer, thin, yellow-coloured layer is raised from the body of the pollen-grain, forming folds upon an inner, thicker, red-brown layer. Both layers coalesce in the wall of the corners. From the side walls of the corners fine teeth project inwards, so that these walls appear as if porous. The apex of the corners is dissolved by the sulphuric acid. The fine threads, binding the pollen-grains together, resist water, sulphuric acid, and potash, and are insoluble also in alcohol. If the grains are treated with 25 per cent. chromic acid, their membrane is soon dissolved, and in all cases the strongly cuticularized portion somewhat earlier than that which is either not, or but slightly cuticularized, which remains for a time as colourless swollen caps on the projecting corners of the plasmic contents. Later on, these also are dissolved; and ultimately even the cobweb threads between the grains do not resist the chromic acid.—From the stigma of an older flower pollen-grains can be

lifted off which have already developed **pollen-tubes**. The formation of tubes takes place commonly only at one corner, or else, of the tubes formed, only one further develops. The membrane of the tube is continuous with the side-walls of the corner; a specially limited **intine** is not present.[7]

Longitudinal sections of the arms of the style of flowers which are past their prime, will show the pollen-tubes developed from the corners of the pollen-grains, traversing the tissue of the style. With magenta the contents of the pollen-grains and tubes will commonly stain deeply, the tissue of the style only very slightly; and hence the pollen-tubes can be traced with great ease, and often in large numbers. Instead of *Œnothera*, an *Epilobium* [Willowherb] or a *Fuchsia* can be used for the investigation.

We will consider still some other pollen-grains of specially characteristic form. The Malvaceæ are distinguished by remarkably large pollen-grains; we examine those of the Hollyhock, *Althæa rosea*. In water these appear globular, opaque, studded with colourless spines. They become very beautifully transparent in carbolic acid and in chloral hydrate, much less so in oil of cloves, still less in oil of lemon. The preparations are best in carbolic acid, so that we will keep to this. Their surface view shows us that the colourless membrane is studded, at approximately equal distances, with large pointed spines. Between these are scattered others, short, blunt, of variable thickness. Regularly distributed circular openings, appearing rose-coloured, traverse the membrane. The basal surface of the membrane is finely punctate. The contents of the pollen-grain appear uniformly finely granular, the nuclei are very difficult to distinguish. The optical section of the grain shows us clearly the form of the large and small spines, and of the canals penetrating the membrane. An exceedingly delicate, but nevertheless existing, **intine** can be traced only as a boundary to the contents; it bulges a little, papilla-like, into the canals of the **extine**. In concentrated sulphuric acid the extine is quickly stained red-brown, and its structure is then very clearly shown.

The pollen-grains of most other Malvaceæ resemble those of *Althæa*. In *Malva crispa*, a hardy annual species not infrequently cultivated, for example, the pollen-grains are shaped just as in *Althæa*, excepting that the spines on the membrane are all alike; between the spines lay scattered the pores or canals; the membrane appears besides finely punctate.

We will use the large pollen-grains of *Althæa rosea*, or of *Malva crispa*, for the purpose of preparing sections through them. Bearing in mind the very trifling difference between the two, we will, however, make use of the latter. Material hardened in alcohol is best for our purpose, and we lay it before use in a mixture of equal parts alcohol and glycerine. We prepare a thick solution of gum, to which we add a small quantity of glycerine, place a drop of this solution upon the smooth end of a piece of elder-pith, and place some pollen-grains on the drop. These are mixed with the gum, and then the drop either allowed to dry in the air, the elder-pith

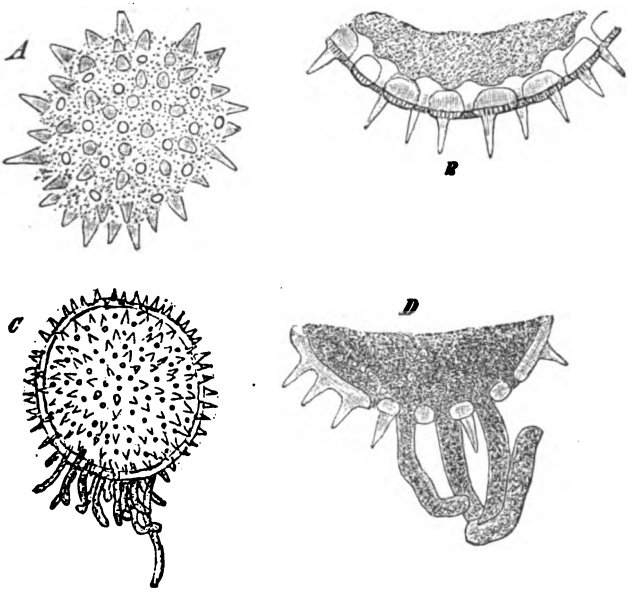


FIG. 1044.—Pollen-grains of *Malva crispa*. *A*, Piece of a grain seen from the surface. *B*, Portion of a section through a grain. *C*, a pollen-grain removed from the stigma, and showing pollen-tubes. *D*, portion of a similar grain in optical section. (*A*, *B*, and *D*, × 540, *C*, × 240).

being placed in a perpendicular position, or else hardened by laying for several hours in alcohol. After this has happened, we prepare delicate sections through the gum with a sharp razor. The sections can be small to any degree, but must be exceedingly thin. They are laid in water or in dilute glycerine, whereby the gum is dissolved and the enclosed sections of pollen-grains are set free. In such sections the structure of the wall of the pollen-grains can be studied in all its peculiarities. Such a section of

Malva crispa (Fig. 104A, *B*), shows the extine to consist most exteriorly of a delicate outer layer beset with spines, next a delicate "rodlet layer," which corresponds with the dots seen in surface view (Fig. 104A, *A*), and a thick homogeneous inner layer, forming convex projections inwards. The intine is swollen under the canal-like openings, elsewhere forms a delicate skin. If such a section is treated with chlorzinc-iodine, the outer layer of the extine and the spines scarcely stain at all, the thickening layer of the extine stains yellow-brown, and the intine blue. The cortex of the pollen-grains swell and colour violet, owing to the presence of the starch-grains, which swell and colour. The unstained as well as the stained sections, provided they are extremely thin and strongly magnified, show us that the pores of the extine are closed externally by a very delicate membrane, the outermost layer of the extine, which passes over them. Delicate sections through the contents in perfectly ripe pollen-grains no longer allow us to recognise the two nuclei which previously were present and readily recognisable. As we may readily assume, they have fallen into small fragments.

If we examine the stigma of an old flower of *Malva crispa* under the simple microscope, or with a strong lens, numerous pollen-grains will be found upon it. On the side turned towards the stigma these will have produced numerous pollen-tubes. If such a grain, the pollen-tubes of which are still short, be removed and examined, we can readily determine that the pollen-tubes come from the canals or pores of the membrane of the grain (Fig. 104A, *C*). This is shown still more beautifully in optical section, after the grain has been made transparent in carbolic acid (Fig. 104A, *D*).

The large pollen-grains of the species of *Cucurbita* have always been specially noticeable for the **valves** which close the places of egress in the extine. In water, yellow oil-drops come off from the surface of the extine, the grains soon evacuate their contents, and the structure of the membrane then becomes clear. The extine is studded with regularly distributed large spines, and between them very numerous small ones. The places of egress are round, the valve is lifted up, either on one side or altogether, by the papilla-like bulging of the intine. The valve has the structure of the surrounding extine, and bears one or more spines. Very good figures are obtained in oil of lemon, less useful in oil of cloves. On the other hand, the figures in chloral hydrate are to be preferred to those in carbolic acid. In a word, the most

favourable clearing medium for each object must be found by experiment. Upon the preparations in oil of lemon and chloral hydrate we can determine, by optical sections, the position of the valve inside the extine, in which it is found wedged with its base somewhat broadening inwards. Under the valve can be seen the bulging of the intine. In sulphuric acid the oil-drops on the extine become blue. The extine slowly becomes brown. The valve is thrust off by the swelling contents. In 25 per cent. chromic acid the entire pollen-membrane is soon dissolved; the intine resists somewhat longer, and, at the moment when the extine disappears, can be followed as a strongly swollen, homogeneous membrane. The pollen-grain has previously emptied itself, whereby the observation of the intine is considerably facilitated. In sulphuric acid, on the other hand, the intine is immediately dissolved, the extine remains, the extruded contents of the pollen-grain are gradually, as in other cases, coloured rose-red.

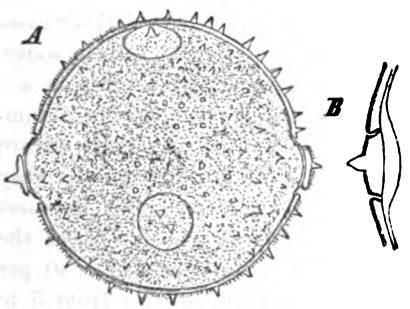


FIG. 104B.—*A*, whole pollen-grain of *Cucurbita Pepo*, seen in surface view, and partly also in optical section; taken from a preparation in oil of lemon (×240). *B*, pollen-grain of *Cucurbita verrucosa*; fragment of a cross-section through the membrane (×540).

The pollen-grain of *Cucurbita* is represented in the adjoining Fig. 104B, in *A*. A complete insight into the structure of the membrane of the pollen-grain can be obtained here also by means of cross-sections, which we can prepare in the same way as described above for *Malva*. The cross-section (Fig. 104B, B) shows clearly the mode of insertion of the valves, that they appertain to the extine, and the structure of the intine.

Of **compound pollen-grains**, which occur alike in Monocotyledons and Dicotyledons, we will first take those of the Ling, *Calluna vulgaris*. The grains here are joined in fours, and usually grouped tetrahedrally. The pollen-membrane shows only slight protuberances, and usually three places of egress for each grain. The various species of *Erica*, *Azalea*, and *Rhododendron* agree in all essentials with *Calluna*. In species of *Acacia*, as in the Mimoseæ generally,[8] the pollen-grains form groups of 4, 8, 12, and 16, and even more cells, but can occur also separately.

In a from 3 to 30 per cent. sugar solution, which contains 1·5 per cent. gelatine, most pollen-grains readily put out tubes, in which protoplasmic movement is beautifully seen. The formation of tubes takes place quite certainly and rapidly in 5 per cent. solution of sugar and 1·5 per cent. gelatine from the pollen-grains of *Pæonia*, *Staphylea*, and even from *Tradescantia* when the pollen-grains are taken from freshly opened flowers. The most favourable objects are perhaps species of *Lathyrus*, *e.g.* Sweet Pea, Everlasting Pea, etc., in 15 per cent. solution of sugar and 1·5 per cent. gelatine. This solution must be freshly prepared, the sowing is best performed in a suspended drop in a moist chamber (see p. 238).

On the artificial production of pollen-tubes a few more words of advice may be given. Though many pollen-grains have a wide range in this respect, for most the degree of concentration of the sugar solution is of vital importance, and therefore it ought to be experimentally determined for each species. A few further cases may be given, spring water being always preferred to rain water. Most species of *Allium* (onion) produce their pollen-tubes in a 3 per cent. solution of sugar, and show active protoplasmic streaming. *Tulipa Gesneriana*, requires from 1 to 3 per cent. sugar; pollen-grains of *Leucojum æstivum* (snowflake) germinate very easily and rapidly in 3 to 5 per cent., and the same with *Narcissus poeticus* (pheasant-eye narcissus); *Convallaria majalis* (lily of the valley) in 5 to 20 per cent.; *Iris sibirica* in from 30 to 40 per cent. The pollinia of orchids produce pollen-tubes in from 5 to 10 per cent. sugar, usually after from 20 to 40 hours. Amongst Dicotyledons the pollen-grains of *Torenia asiatica* germinate very readily, producing pollen-tubes in 10 per cent. sugar solution after about two hours, and the tube grows so rapidly that, with strong magnification, its tip can be seen to move across the field of view. Species of *Gloxinia* behave in much the same way in 10 per cent. solution; *Papaver* (poppy), 1 per cent.; *Viola tricolor* (pansy), 30 per cent.; *Ampelopsis hederacea* (Virginia creeper) 20 to 30 per cent. In all these cases 1·5 per cent. of gelatine is added.—The pollen-grains invert the cane-sugar of the culture fluid, the invertin being present in the grain prior to germination. The pollen grains likewise contain diastase, and are capable therefore of dissolving starch-paste if present in the culture fluid.

NOTES TO CHAPTER XXVIII.

[1] On Stamen and Pollen compare v. Mohl, *Ueber den Bau und die Formen der Pollenkörner*, 1834. Fritsche, *Ueber den Pollen*, *Mém. de sav. étrang.* 1836. Naegeli, *Zur Entwicklungs. d. Poll. bei den Phanerogamen*, 1842. Schacht, *Jahrb. f. wiss. Bot.*, Bd. II., p. 109. Warming in *Hanstein's bot. Abh.*, Bd. II., Heft. II. Strasburger, *Befr. und Zellth.*, p. 15, and *Bau der Zellhäute*, p. 86. Elfving, *Jen. Zeitschr. f. Naturw.*, Bd. XIII., p. 1 [trans. in *Quart. J. of Mic. Science*, 1879]. Goebel, *Grundz. der. Syst. Bot.*, p. 398. Luerssen, *Grundz. d. Bot.*, III. Aufl., p. 359; *Med. Pharm. Bot.*, Bd. II., p. 198. Prantl, *Lehrb. der Bot.*, III. Aufl., p. 192 [English trans., by Vines].

[2] Sachs, *Bot. Zeitung*, 1862, p. 242.

[3] Compare *Leclerc du Sablon*, *Annales des sc. nat.*, *Botanique*, VII. Sér., Vol. I. p. 97, 1885.

[4] Warming, in *Hanstein's bot. Abh.*, Bd. II., Heft. II. Goebel, *Grundzüge*, p. 409.

[5] Compare herewith, Elfving, *Jenaische Zeitsch.*, Bd. XIII., p. 12 [English translation as above].

[6] Strasburger, *Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen*, 1884, p. 5.

The differential coloration of vegetative and generative nucleus is in general more strongly marked than in *Tradescantia*.

[7] Strasburger, *Bau der Zellhäute*, p. 95, where the development is given.

[8] Rosanoff, *Jahrb. f. wiss. Bot.*, Bd. IV., p. 441. Engler in the same, Bd. X., p. 277. The other literature is there given.

CHAPTER XXIX.

THE GYNÆCIUM OF ANGIOSPERMS.

MATERIAL WANTED.

Fading flowers of Larkspur, *Delphinium Ajacis*. Fresh; or in alcohol. *Or*, the same of the Hellebore, *Helleborus* sp.

The same of the Flowering Rush, *Butomus umbellatus*.

The same of some liliaceous plant, *e.g.*, Tulip, Hyacinth, *Lilium*, *Hemerocallis*, *Yucca*.

The same of a *Primula*, *e.g.*, Primrose, Cowslip, Auricula, Polyanthus, or of *Lysimachia*, or *Anagallis*.

Withering flowers of an orchid.

Full-blown flowers of Monkshood (*Aconitum Napellus*, etc.).

The same of the Bird's-nest Rape, *Monotropa hypopitys*. Fresh only. *Or*, the same of some sp. of *Pyrola* (Winter-green). Or some orchid. *Or Gloxinia*. Fresh. Alcohol-material will do.

Full-blown flowers of *Torenia Asiatica*. Fresh. Alcohol-material will do.

The same which we have ourselves pollinated from 36 to 48 hours previously.

LET us first obtain a general idea of the structure of the **Ovary**.[1] For this purpose one of the Ranunculaceæ is very well suited, *e.g.* *Delphinium Ajacis*, the Larkspur of the gardens. We choose an old flower, from which the petals and stamens are easily removed, and observe three **pistils** * left standing in the central position. Even by superficial observation we can distinguish upon the pistil the lower, green, swollen portion—the **ovary**, and the thin part, here rose-coloured, into which the ovary narrows above—the **style**. This last ends with the **stigma**, which in this case is not specially

* It is high time an end was put to the confusion existing in systematic works as to the use of this term. A *pistil*, I define as a distinct ovary, of one or more cells, and composed of one or more conjoined carpels, with its stigma or stigmas, and, if present, style or styles. A *gynæcium* is the whole female part of the flower, consisting of one or more such pistils. Pistil and gynæcium may thus be synonymous, as when there is but one pistil, but are not necessarily so. This may not be throughout consistent, but it is at least clear. [ED.]

delimited, and merely ends the style.—We now prepare cross-sections through all three ovaries together, and examine them with a low power, or with the addition of a little potash. The cross-section (Fig. 106) shows us a single cavity [**cell** or **loculus**] in each ovary. Apparently it is a single fertile leaf, or **carpellary leaf**, which forms each such ovary. We can conceive the carpellary leaf folded inwards and its edges here grown together. To such an origin points, moreover, the **ventral suture**,* which we find, in fact, in the median plane of the ovary on its side turned towards the middle of the flower. Such an ovary, composed of one fertile leaf, is **monocarpellary**; when a number of such monocarpellary ovaries are combined in a single flower, as is the case in our example, the flower is said to be **apocarpous** or **polycarpous**. The ovaries are here free to their base, and inserted upon the **floral axis**, *i.e.*, they are **superior** or **free**. The entire female sexual apparatus of the flower may consist of one or of numerous pistils, and is designated the **gynæcium**. Our cross-section shows clearly the groove on the ventral side; and with stronger magnification we can, at this place, follow the external epidermis through the entire thickness of the wall, and see it continue into the epidermis of the ovarian cavity. It is interesting that this inner epidermis also possesses **stomata**. The wall of the ovary is traversed by a number of fibro-vasal bundles, of which most appear on the dorsal side, and some near the edges of the carpel on the ventral side. The edges of the carpellary leaf are a little swollen, and form, on the cavity side, the **placentæ** (*p*). From these the **ovules** (*s*), corresponding with the number of placentæ, arise in two rows. With the ovules we shall concern ourselves later on, and for this purpose we put our preparation on one side.

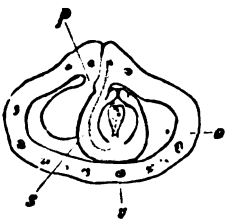


FIG. 106.—*Delphinium Ajacis*. Cross-section through an ovary. o, wall of the ovary; v, fibro-vasal bundles in the wall; p, placenta; s, rudimentary seed [ovule] (× 18).

Instead of *Delphinium*, and available so early in the year as

* While there can be no sound objection to the use of the term *ventral suture* to imply this union of the margins of the leaf, there is no reason whatever for retaining the absurd term *dorsal suture* for the midrib of the carpellary leaf. The term "dorsal" is however of advantage in connection with dehiscence, and it would greatly facilitate a much-needed simplification of the methods of classification to speak, for instance, of the "ventral" dehiscence of a follicle, the "dorsi-ventral" dehiscence of a legume, and so on for the various kinds of capsule. [ED.]

February, we can use the ovaries of the stinking Hellebore, *Helleborus fœtidus*. They agree in all essentials with the above. Sections taken through flower-buds at various stages will show moreover the method of union of the margins of the carpellary leaf. Some of the sections will show these margins unjoined but in contact; in others, the epidermal cells of one thickened margin will be seen to grow out, papillately, between the similar cells of the other margin, so as to "dovetail" the edges together. Sections of the same young flower-buds may show the division of the pollen mother-cells into tetrads. For earlier examination still the Christmas Rose, *H. niger*, may be used.

In the flower of the Flowering Rush, *Butomus umbellatus*, as in *Delphinium*, we find a number of ovaries—always six; but these ovaries are free only in their upper half; in the under half they have grown together laterally, and cannot be isolated uninjured. The style is very short, and bears the stigma on its upper edge. We prepare cross-sections through the free and the combined portions of the ovaries. The figure of the free upper part is, from the point of view of the carpellary leaf, the same as in *Delphinium*; the individual carpellary leaves remain distinguishable from one another to their base, but in the lower part it is no longer possible upon the cross-sections to separate the individual carpellary leaves intact. In *Butomus* we have therefore an intermediate stage between **apocarpous** and **syncarpous** flowers; and this example is suitable to introduce us to the **multilocular** ovary, composed of more than one carpellary leaf. Besides this, we find another novelty in *Butomus*. The ovules arise not only from the edges, but rather, the median line excepted, from the whole inner surface of the carpellary leaf; they have **superficial placentation**. The entire walls are covered with ovules, and functionate as placentæ. At the place of insertion of each ovule a fine fibro-vasal bundle is to be seen, which provides for the ovule. They are branches of the stronger, larger, fibro-vasal bundles, lying deeper in the tissue.

The ovule of Liliaceæ is on an **axile placenta**; we select, with like results, the Tulip, Hyacinth, a Lily, or *Hemerocallis* for examination. In the Tulip the three stigmatic lobes are sessile upon the ovary, without style. In the Hyacinth the style is short, the stigma small, slightly trifid. In *Lilium* the style is long, the stigma tripartite. In *Hemerocallis* the style is very long, the stigma likewise tripartite, but very small. Cross-sections show

us a **trilocular** ovary, composed of three closed carpellary leaves which have grown together. Here neither laterally nor in the middle is a limit between the tissues of the individual carpellary leaves to be recognised; and a single continuous epidermis covers the exterior of the whole structure. In *Yucca*, another genus of the Liliaceæ, the limits of the three carpellary leaves are marked externally by grooves, and internally by narrow radial pear-shaped cavities in the tissue, each cavity being lined by a distinct epidermis. Three carpellary leaves therefore form here a syncarpous, trilocular ovary. Each of the three carpellary leaves combined into this trilocular ovary bears, corresponding to its two edges, two rows of ovules; *i.e.* the placentæ lie here in the inner angles of the loculi or cells of the ovary. The placentation is therefore **marginal**, as in *Delphinium;* but as they arise from the angles of the cells, and therefore in the centre, it is specially designated **central** or **axile placentation.**—Cross-sections through the style of *Hemerocallis* show us in it a central triangular passage, the **pollen-canal.** Three fibro-vasal bundles are distributed at the three angles of the pollen-canal. A longitudinal section through the apex of the style, and therefore through the stigma also, shows us the surface of this latter grown out into long papillæ. This phenomenon is very general upon stigmatic surfaces; *Hemerocallis* however offers still another interesting condition, in that the cuticle of the papillæ is raised up by the formation of slime or mucus. This cuticle is spirally striate, and in accordance with this its upheaval follows a spiral line. At length the cuticle is entirely loosened from the inner layer of the membrane, and disappears from the papillæ.—The other Liliaceæ likewise show a hollow style; in most cases, on the other hand, the style is solid, but filled either with cells easily passing out of lateral union, or else provided with swollen side-walls, between which the pollen-tubes can easily grow downwards.

Another free or superior ovary exists in the flowers of the species of *Primula* [Primrose, Auricula, Cowslip, etc.]. These are **dimorphous**, *i.e.*, have short-styled and long-styled ovaries, and stamens inserted high up or low down upon the tube of the corolla. A median section taken through the ovary shows us that the floral axis is prolonged into the cavity of the ovary, and here enlarges into a mushroom-like swelling. In the middle this swelling projects, papilla-like, into the pollen-canal of the style. The entire surface of this swelling is covered with ovules. We

have here a **free-central placenta**. The wall of the ovary is in no way connected with this placenta. We can be quite convinced of this by cross-sections in which the wall of the ovary appears as a free ring around the central placenta, or, if an equatorial incision is made round the ovary, the style and upper part of the ovary can be lifted off like a cap from the mass of ovules, and the prolongation of this central swelling will be withdrawn from the pollen-canal. Wanting also in the ring are the points of separation which enable us to determine the number of carpellary leaves concerned in forming the ovary; these however are assumed to be five, from the point of view of the numerical symmetry of the other floral parts, and from the circumstance that in many Primulaceæ the fruit-capsule dehisces at its apex with five teeth. In *Primula* itself the number of the teeth with which the capsule opens is undetermined.—Instead of *Primula*, species of *Lysimachia* [Loose-strife, Creeping Jenny, Money-wort, etc.,] or of *Anagallis* [Scarlet Pimpernel, Bog Pimpernel, etc.,] can be used with the same results; they all bear their ovules on a free central placenta.

Let us examine now an **inferior** [**adherent** or **adnate**] **ovary**, selecting first that of *Epipactis palustris*, or of some other orchid. The brown ovary lies below the point of insertion of the other floral parts. We select for cutting a young rudimentary fruit, upon which the petals have already begun to go brown. Cross-sections are very instructive, and show us a unilocular ovary, which bears equidistantly upon its wall three pairs of placentæ. The placentæ repeatedly divide at their inner edges, and bear a great number of ovules. The wall of the ovary has on its outer side six projecting ribs, of which three correspond to the places of insertion of the placentæ; three specially strong ones alternate with these places of insertion. Each rib is traversed by a fibro-vasal bundle, or a complex of fibro-vasal bundles, besides which a small bundle lies at each place of separation of two placentæ. In a superior ovary, the cross-section of which should agree completely with that here described, we should in no way scruple to consider the ovary as composed of three carpellary leaves, and to look upon the pairs of placentæ as arising from the conjoined edges of two adjoining carpellary leaves. The three ribs which alternate with the lines of insertion of the placentæ, we should take for the midribs of the three carpellary leaves. As we have here, however, an inferior fruit, the matter is not so simple. We can either conceive that the inferior ovary consists

of the hollowed floral axis, and is only closed above by the carpellary leaves, that from these latter, however, the placentæ grow downwards into the hollowed floral axis; or we can consider that the carpellary leaves and hollowed floral axis have grown together, and that in the wall of the inferior ovary, therefore, the outer portion appertains to the stem, the inner to the carpellary leaves. This latter theory is decidedly to be preferred; it has, however, no other than a phylogenetic, or evolutionary, value; *i.e.*, we conceive that in course of time the inferior ovary has so arisen.* In point of fact, however, the anatomical and physiological *data* for such a conception are wanting, and we must therefore be contented with stating that the structure of this inferior ovary is not different from that of a polycarpellary, unilocular, superior ovary.—If ripe fruit-capsules of *Epipactis* are at our disposal, we shall find in these, as in most other Orchideæ, that the wall of the capsule dehisces by six longitudinal clefts. The six bands separating the clefts remain joined at the base and at the apex of the capsule. Three of them are broader and fertile, three are narrower and sterile. The three sterile correspond with the three mid-ribs, which we saw in the cross-section of the ovary; the three fertile bands bear in their middle the placentæ.[a]

We will now endeavour to become acquainted with the structure of the ovule, and at the same time turn our attention to the processes of fertilization in Angiosperms. In order to become acquainted with the individual parts of the ovule, we first prepare cross-sections through the ovary of the Monkshood, *Aconitum Napellus*, or of some other species of *Aconitum*. We select a flower in full bloom, strip off the other parts of the flower, and then cut through the three ovaries together. Care should be taken that the sections are taken correctly at right-angles with the long axis of the individual ovaries. The number of the sections must be considerable, so as more probably to cut an ovule correctly. We glance over the sections, and select those which appear likely. In case the section is not delicate enough, we can help matters with a little potash. The figures are almost identical with those which we have just examined in *Delphinium;* but in the structure of the integument of the ovule there is a slight difference, which induces us to give *Aconitum* the preference now. If an ovule is cut centrally, it appears as in the adjoining Fig. 107. The ovary

* A plant of great interest in this connection is the Californian poppy, *Eschscholtzia Californica*, now so commonly grown in gardens. [Ed.]

[a] See note on page 337.

is **monocarpellary**, the ovule arises from a **marginal placenta**. It is inserted thereon with a stalk, the **funiculus** or **funicle** (*f*); the free part of this is very short, the rest of it is grown to the ovule, forming upon it the **raphe** (*r*). In the body of the ovule we distinguish first of all the inner conical mass of tissue as the so-called nucleus of the ovule—the **nucellus** (*n*). This corresponds with the **macrosporangium** of the Vascular Cryptogams. The nucellus is encased in two **integuments**, an inner (*ii*) [originally called the "secundine" by Mirbel; but, as it is developed before the other, more recently known as the "primine"], and an outer (*ie*) [originally called the "primine"; but, as it is developed after the other, more recently called the "secundine"]. The inner is developed on all sides to the base of the nucellus, the outer is wanting on the side of the raphe, in that it joins on both sides to the funiculus. The inner integument leaves a narrow canal free between its upper edges, which extends to the nucellus; this canal is known as the **micropyle**. The funiculus is traversed by a fibro-vasal bundle, coming from the placenta, which in many, but not, however, in all, cases can be traced to the base of the nucellus. The tissue adjoining the base of the nucellus, here distinguished by its brighter coloration, is known as the **chalaza** (*ch*). In the long axis of the nucellus is noticeable a larger cell, forming quite a cavity; this is the **embryo-sac** (*e*). At its base can be seen some globular cells, which in *Aconitum* (and Ranunculaceæ generally) are very strongly developed—the **antipodal cells** (*a*). In specially favourable cases we can determine that they are three in number. In the apex of the embryo-sac we can also see a small cell, which, however, is only recognisable in perfectly median sections; it is the egg-cell or **oosphere**, sometimes called the embryonic vesicle or germinal vesicle (*o*). The ovule as a whole is distinguished as **anatropous**, *i.e.* turned back, because the body of the ovule does not lie in direct continuation of the funiculus,

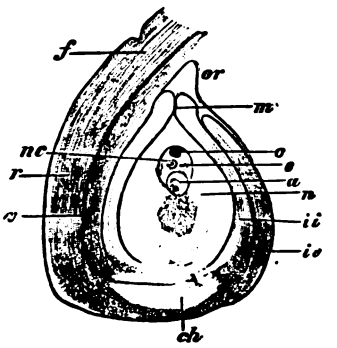


FIG. 107.—*Aconitum Napellus*; median longitudinal section of an ovule. *f*, funiculus; *r*, raphe; *v*, fibro-vasal bundle of the funiculus; *i.e.*, outer integument; *ii*, inner integument; *n*, nucellus; *ch*, chalaza; *e*, embryo-sac; *a*, antipodal cells; *o*, the oosphere; *nc*, nucleus of the embryo-sac; *m*, micropyle; *or*, wall of the ovary (× 53).

but appears laid by the side of it, with one side grown to it, and the micropyle turned to the base of the funiculus. This form of ovule is by far the most common in Angiosperms. If we now compare our preparation of *Delphinium* (Fig. 106) with that of *Aconitum* (Fig. 107) we shall see that the structure of the ovary and ovule in the two cases is quite identical; the distinction only is that in *Delphinium*, as very commonly in Ranunculaceæ, the two integuments of the ovule are blended together.

In order to prepare sections of the ovule of *Aconitum*, we can separate one from the ovary, and cut it singly between the thumb and fore-finger, in the method already known to us. If the ovule is correctly arranged between the fingers, we shall in this way more rapidly obtain true median sections. In this and in other like cases the ovule may, with advantage, be first embedded in glycerine-jelly or in celloidin (celluloidin), and then cut. The glycerine-jelly must be tolerably firm, *i.e.*, must contain a comparatively large proportion of gelatine. In celloidin only alcohol material can be embedded. We pour the solution of celloidin* into a small box made of writing paper, and immerse the ovule in it. The celloidin is then allowed to stand in the air till it has acquired a firm skin, when it is laid in 82 p.c. alcohol. Here, after some hours, it acquires the consistence of cartilage, remaining transparent. Celloidin and object are cut together, and the sections laid in glycerine or glycerine-jelly, without its being necessary to remove the celloidin. The sections can be stained with carmine or with logwood (hæmatoxylin), but not with aniline colours, as these latter colour the celloidin as well. If the celloidin has been procured in cakes, it must be dissolved before use in equal parts of ether and absolute alcohol. In order to make ovules which are to be embedded in glycerine-jelly or in celloidin still more visible, they can be previously stained with watery logwood; the ovules must then, however, after previous washing in water, be again dehydrated in absolute alcohol, before being placed in the celloidin. Objects which, in order to make them available for section-cutting, must be permeated with celloidin, are first treated with dilute solution of celloidin, in which the object must lie often for days before it is transferred to, and embedded in, the thick celloidin solution.

* To be obtained of Dr. Grübler, in Leipzig, Dufour-strasse, 17. In cakes at about 3*s.* each, or in solution at 11*s.* the kilogramme. Also of Messrs. Southall Bros. & Barclay, manufacturing chemists, Dalton St., Birmingham. [Ed.]

We will now take in hand the study of the interior of the **embryo-sac**. The most favourable object for this is *Monotropa Hypopitys*, the Bird's-nest Rape, a pale yellowish parasite, found chiefly under beech and fir trees.[2] This plant is so favourable for the otherwise difficult investigation of the embryo-sac that no pains should be spared, if possible, to obtain it.* It flowers in July and August, and must be examined fresh, as in alcohol it becomes brown and opaque. The plant bears carriage very well, and can be preserved healthy for a very long time in a glass of water. Supplies may in this way be obtained from a distance.

With *Monotropa* agree the various species of *Pyrola*, or "winter-green," likewise Ericaceous plants, excepting that their ovules are smaller. About a dozen species of *Pyrola*, all hardy herbaceous perennials, can be readily enough cultivated in gardens, either from seeds or division of the roots, selecting for the purpose a shady border, with a sandy peat soil. The cross-section through the under part of the ovary shows this to be four-celled, 5-celled in *Pyrola*. The placentæ are strongly swollen, and bear on their surface very numerous, slender, closely serried ovules. The two halves of the placenta in each cell are removed to some little distance by a radial line of separation. In the upper part of the ovary these lines of separation extend to the centre, and there adjoin one another. We see then four strong pairs of placentæ, each placed on the centre of a partition wall, which appertain to the two neighbouring loculi; the pairs are easily separated from one another with the needles. We get the ovules for our investigation by removing a portion of the wall of the ovary with the forceps, and stripping off the ovules with the needles from the placenta thus exposed. We place them in pure water, or in 3 p.c. solution of sugar, in which the ovules remain longer unchanged. We take this material from an oldish flower in which the stamens have already dehisced, so that we shall find the ovules in part ripe and not yet fertilized, in part already fertilized. Between the ovules we come often upon pieces of pollen tubes. The receptive ovule has the appearance of the adjoining fig., 107. It is transparent, and can be focussed for optical section. We recognise in it an **anatropous ovule**, with but one integument (*i*). The whole interior of the ovule is filled by the **embryo-sac**; we miss the nucellus, which, during the develop-

* It is hardly likely to be regularly obtainable in England, so that the alternative plants referred to later on must be relied on for material. [Ed.]

ment, is pressed back by the embryo-sac. The apex of the embryo-sac is occupied, as we can clearly see, by three cells. These three cells form the **egg-apparatus** or **germinal apparatus.** They are not of equal value. The two upper are the assisting-cells, or **synergidæ** (Fig. 108, *B*); that more deeply inserted, is

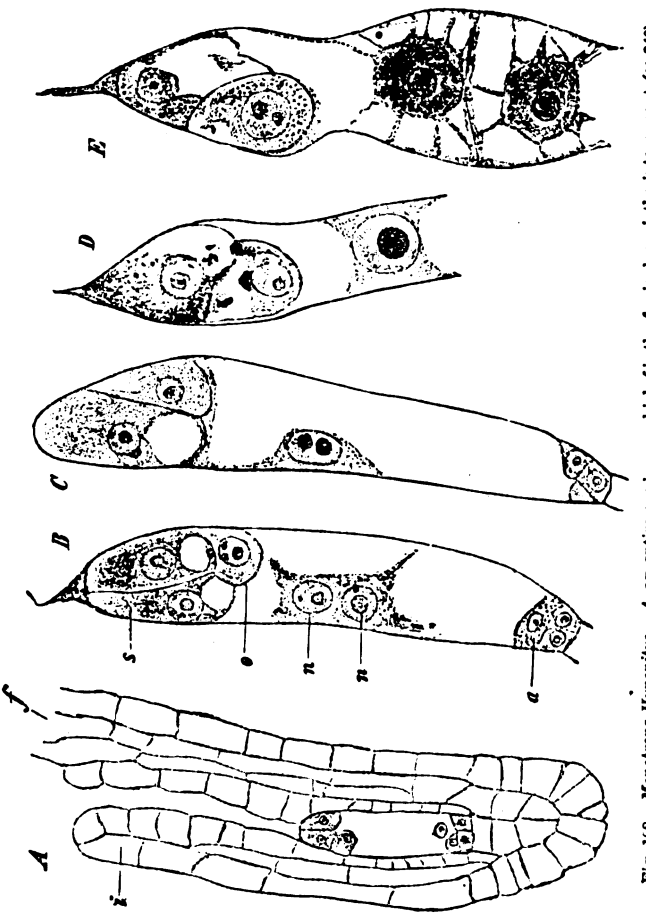


Fig. 108.—*Monotropa Hypopitys*. *A*, an entire ovule, on which *f* is the funicle; *i*, the integument (× 240). *B* and *C*, the entire embryo-sac, in which are *s*, the synergidæ; *o*, the oosphere; *n*, the nucleus of the embryo-sac; *D* and *E*, upper parts of the embryo-sac; in *E* the first division for the formation of endosperm. (B to E × 600.)

the **oosphere** (*o*) [germinal vesicle, embryonic vesicle]. The synergidæ, as can be easily seen, have in their lower part a vacuole, are filled above with protoplasm, and here contain the **nucleus.** The oosphere inversely has its cavity above, and below the main mass of its cell-protoplasm, and the nucleus. Both

synergidæ are not always seen, as one can cover the other (Fig. 108, *C*). At the base of the embryo-sac the **antipodal cells** can usually be recognised without difficulty, and we can count that three also of these are present. In the interior of the embryo-sac is usually found a **nucleus**, with a **nucleolus** (Fig. 108, *A*); but in other cases there are two nuclei (*B*) or a nucleus with two nucleoli (*C*); and we judge from this, that the one nucleus which we always ultimately find arises from the union of two. Ovules, the fertilization of which has already commenced, can be recognised by the changes which the synergidæ have undergone. These appear strongly refractive, both or only one being thus modified. It is then certain that a pollen-tube has penetrated to the embryo-sac, and if it is not easy to see it in the interior of the micropyle, it is still not difficult to recognise the piece torn off from it in the preparation and projecting beyond the micropyle. The apex of the pollen-tube, however, has penetrated to the synergidæ, and the protoplasm of the pollen-tube between the synergidæ to the oosphere. With careful examination we may happen, in oospheres which border on synergidæ that are thus changed, to find two nuclei (*D*), one larger, the original nucleus of the oosphere, and close by it also a smaller, the **spermatic-nucleus**, which has penetrated from the pollen-tube. This latter increases quickly in size. We can find stages of conjugation between the **oo-nucleus** and this **spermo-nucleus**, and afterwards see only one **embryo-nucleus**, with two unequal nucleoli, of which the smaller arose from the spermatic-nucleus (*E*), and ultimately an embryo-nucleus with only one nucleolus. While the oosphere is being fertilized, the highly refractive masses of substance in one or both synergidæ diminish; they are apparently used for the nourishment of the **oospore**. At the same time with these changes in the egg-apparatus the formation of **endosperm** has commenced in the cavity of the embryo-sac; *i.e.*, we see the embryo-sac divided by walls. The endosperm-formation here, therefore, takes place by **cell division**. In other equally frequent, even more frequent, cases, the nucleus of the embryo-sac and its descendants at first divide **free**; and only at a later stage of the development the formation of partition-walls between these nuclei commences. The process, as we have it here, takes place in general in such embryo-sacs as show slow, and on the whole inconsiderable, increase in size. Where, on the other hand, the embryo-sac increases very rapidly in size after the fertilization of the egg-cell, there **nuclear division**

without cell-division first takes place, and **cell-formation** *i.e.*, the formation of the partition-walls, first begins when the embryo-sac is approximately fully developed.—In consequence of fertilization the oospore has acquired a delicate cellulose membrane, and soon begins to elongate into a sac, and after some time penetrates with its apex into the body of the endosperm, where the apex of the sac produces a few-celled **embryo**, the rest of the sac forming the **suspensor**. — We have thus far examined these ovules only in pure water or in sugar-solution; if we wish to see the nuclei come out clearly, we must treat the ovules with two per cent. acetic acid. In this way we obtain very sharply-defined structures in most ovules, and at the same time fix the stages of division of the nuclei, although into these processes we do not propose at present to go more deeply. Staining media cannot be recommended, since they stain also the nuclei in the integument, and in this way injure the view into the interior.

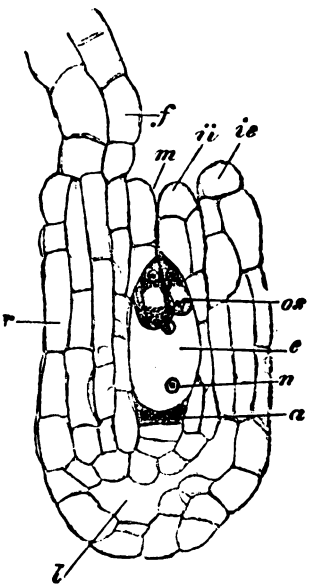


Fig. 109.—*Orchis pallens*. Receptive ovule. *os*, egg-apparatus; *ii*, inner, *ie*, outer integument; *l*, air-cavity The other letters are as in the previous figures (× 240).

Instead of *Monotropa* various Orchids (*Orchis* and other genera) can serve for this investigation. Fertilization takes place in these a good while after pollination, and in ovaries which are already greatly enlarged. These are cut open, ovules removed with the needles from a placenta, and transferred to water or three per cent. solution of sugar. We can, without further steps, inform ourselves as to the structure of the fully-formed ovule (Fig. 109); this is very like to that of *Monotropa*, but there are two integuments, as commonly in monocotyledons, and an air-cavity in the neighbourhood of the chalaza. This air-cavity makes observation more difficult if it is filled with air, and this latter also penetrates between the integuments. The ovule in water or in three per cent. sugar solution, must therefore be freed from air under the air pump. Often even a slight pressure upon the cover-glass

serves to remove the most disturbing air, found between the integuments. The nucellus in the Orchideæ also is quite displaced by the embryo-sac; as a relic of the nucellus a strongly refractive cap of substance is still to be seen at the apex of the embryo-sac. The egg-apparatus (*os*) is constructed as in *Monotropa*, only that the oosphere is less deeply inserted. The antipodal cells are not to be seen; in their place is a strongly refractive substance, in which lie, in fact, three nuclei, recognisable, however, with great difficulty. The pollen-tube can, more easily than in Monotropa, be traced to the synergidæ; the changes which the synergidæ undergo are the same. The two nuclei, moreover, are again found in the fertilized oosphere. Endosperm is in general not formed in the Orchideæ.

In default of *Monotropa* and of Orchidaceæ transparent ovules for investigation are provided by various Gesneraceæ,[4] and, above all, the large-flowered *Gloxinia hybrida* of the gardens. The ovule, having only one integument, is so far transparent that the egg-apparatus is clearly visible. It shows the two synergidæ, and in this case flask-shaped oosphere. Under some circumstances two oospheres can be present. The embryo-sac in its upper part is swollen, but narrows suddenly below; the antipodal cells in the lower end are not distinguishable with certainty.

One of the most favourable plants for the study of fertilization is however the Scrophularineous plant *Torenia Asiatica*.[5] This stove-evergreen from the East Indies is now cultivated very generally in gardens, and bears flowers the whole year through. It is distinguished in that its embryo-sac grows upwards into the micropyle, and hence the whole egg-apparatus appears without any other covering than the wall of the embryo-sac. Cross-sections through the superior, elongated ovary show this to be two-celled; the two axile placentæ project as pads into the loculi. They are covered with numerous ovules. For the purpose of observation we remove a wall of the ovary, and strip off the ovules from the placenta, best under the simple microscope. We observe them with advantage in a 3 p.c. solution of sugar. The ovules are anatropous, or, more correctly, somewhat campylotropous, for the embryo-sac and the integument are bent in their upper part (Fig. 110, *A*). The free part of the funiculus (*f*) of the ovule is pretty long. There is only one strong integument. The embryo-sac (*e*) projects with its upper end out of the micropyle. This protruding part is convexly swollen and pointed at its

apex; it lies against the funiculus. It is difficult to follow the embryo-sac in the interior of the ovule, but by running in a little potash we can, during its commencing action, convince ourselves that it immediately adjoins the integument, is first very narrow,

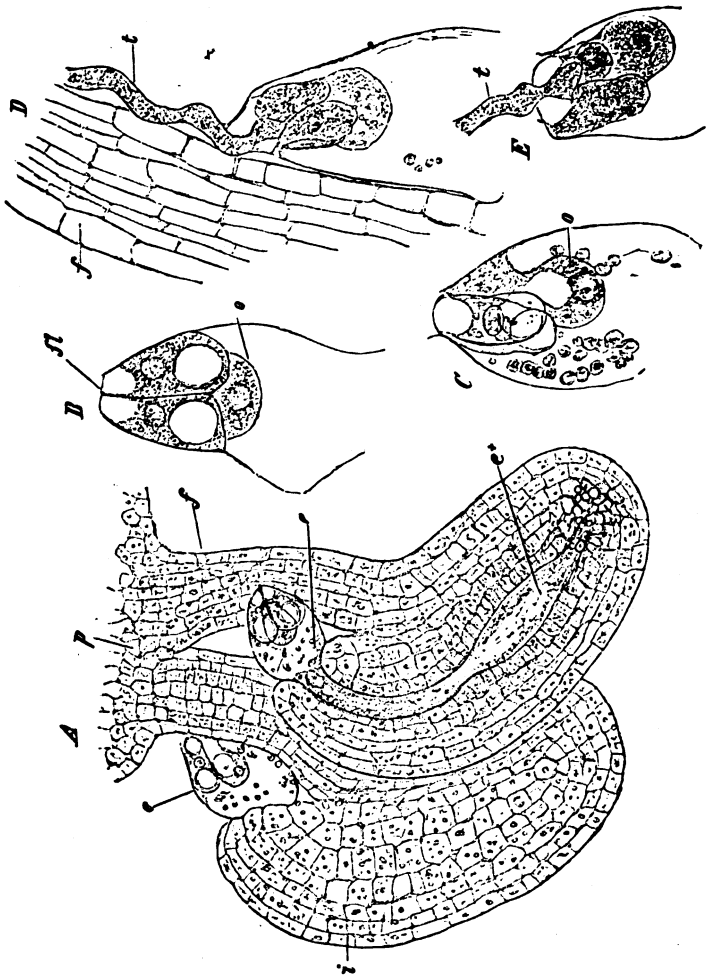


FIG. 110.—*Torenia Asiatica*. *A*, two ovules on the placenta (*p*); *e*, the free apex of the embryo-sac; e^*, its broadened part in the interior of the ovule; *f*, the funiculus; *i*, the integument (× 240). *B* and *C*, free apex of the embryo-sac before fertilization; *fl*, cape of the synergidæ (filiform apparatus); *o*, oosphere. *D* and *E*, during fertilization, *D* with a part of the funiculus *f*; *t*, pollen-tube. (*B*–*E* × 600.)

then swells somewhat spindle-shaped, and (e^*) again narrows at the base. Our preparations in sugar solution show, in the free apex of the embryo-sac, the two synergidæ and the oosphere; once more therefore, as always, three cells form the egg-apparatus.

According to the position of the preparation two synergidæ are to be seen (Fig. 110, *B*), or one covers the other (*C*). At the apex of each synergida we notice here a homogeneous, strongly refractive cap, sharply defined against the finely granular portion behind; this is the so-called **filiform apparatus**. If such a preparation is treated with chlorzinc iodine, the caps of the synergidæ are seen to colour blue. They consist therefore of cellulose. The other substance of the synergidæ and of the oosphere colours yellow-brown. Careful examination shows that the membrane of the embryo-sac is open over the caps of the synergidæ (*B*, *C*). The filiform apparatus therefore now forms the stopper to the opening of the embryo-sac. This apparatus is very widely distributed, especially amongst monocotyledonous plants, and projects often very far out of the embryo-sac. The longitudinal striation, which they very commonly show, arises from fine pores filled with protoplasmic contents.—We turn again to our preparation lying in water or in sugar solution, and further determine that here also the distribution of the contents in the synergidæ and the oosphere is entirely the same as in *Monotropa* and *Orchis* (*B*, *C*). In the synergidæ the nuclei lie in the upper, the vacuole in the lower part; in the oosphere this is reversed.—If we wish to study the process of fertilization in *Torenia* we must pollinate the flowers for this purpose. From pollination to fertilization thirty-six hours elapse, so that we must renew our observations after from a day and a half to two days. As before, we free the ovules from the placenta, but as carefully as possible, under the simple microscope, in order to remove the largest possible proportion of the pollen-tubes. These are followed here, with the greatest possible ease, to the apex of the embryo-sac, between the caps of the synergidæ and right up to the oosphere (*D*, *E*). We see that the pollen-tubes conducted by the placentæ are still further led by the funiculi till they attain the apex of the embryo-sac. A direct influence makes itself felt at the same time from this latter, which effects the direction of growth of the apex of the pollen-tube. It can be assumed that the synergidæ secrete a definite substance which acts as a stimulus upon the pollen-tube. The caps of the synergidæ, on account of their soft consistence, oppose little resistance to the escape of the secretion. Where the caps of the synergidæ are specially strongly developed, they appear besides traversed by very fine canals, which conduct the secreted substance outwards. The synergidæ in *Torenia*, as in others, become dis-

organized after the entrance of the pollen-tube, and take on the strongly refractive appearance already known to us. For the study of the further stages of development of the embryo, etc., this object is not favourable.

One of the easiest plants in which to see the passage of the pollen-tube into the micropyle is a speedwell, *Veronica serpyllifolia*, very common in shaded grass and in hedgerows. Take a flower from which the corolla has just fallen, place the ovary on a slide in a drop of water or of 3 per cent. sugar solution, and dissect out the ovules under the microscope or under a simple microscope. If the ovules are carefully separated, and a cover-glass put on, some of them will probably be found to show the pollen-tube in the micropyle.

NOTES TO CHAPTER XXIX.

[1] Goebel, *Grundzüge d. Syst., etc.*, p. 417. Lürssen, *Grundz. der Botanik*, p. 356. *Med. Pharm. Bot.*, Bd. II., p. 244. Prantl, *Lehrbuch der Botanik*, 4th edit., English edition by Vines, p. 204.

[2] Strasburger, *Befruchtung und Zelltheilung*, pp. 34, 35.

[3] The same, p. 55.

[4] The same, p. 54.

[5] The same, p. 52.

[*Note to page* 327.]

[6] A multicellular inferior or adherent ovary we find in *Œnothera biennis* (evening primrose), or any other *Œnothera*, *Epilobium*, or *Fuchsia*. The ovary here often lies considerably below the point of insertion of the flower. The cross-section shows four cells or loculi. The placentæ arise from the inner angles of the cells; they project somewhat into the cavity of the cells, and bear two or three rows of ovules. The middle line of each cell is represented by a depression. At this point lie weak vascular bundles, a strong one externally and a weaker one internally, before the partition walls. The inner one, by means of horizontal branches, which the cross-section often lays bare, is connected with the bundles which occupy the central tissue between the four cavities. These for their part, support the placentæ. The wall of the ovary contains numerous raphides which, set free from their cells, lie scattered over the whole section.

[*Note to page* 333.]

[7] Fertilization of orchids takes place in from 3 to 10 days after pollination, according to the species.

CHAPTER XXX.

STRUCTURE OF THE SEED OF ANGIOSPERMS.

MATERIAL WANTED.

Flowers and seed-pods of various ages of the Shepherd's Purse (*Capsella Bursa-pastoris*). March to November. Fresh, also in alcohol.
The same of the Water-Plantain (*Alisma Plantago*). June to August.
Ripe seeds of the Castor Oil plant (*Ricinus communis*).
Ripe grains of Wheat. Fresh.

WE will now endeavour to make ourselves acquainted with the structure of a ripe **seed**, and to give especial attention to the **embyro** which it contains. As a comparatively favourable plant, we select one of the Cruciferæ, *Capsella Bursa-pastoris*, the shepherd's purse, a plant which has been very commonly made use of for embryological studies.[1] Its seed is comparatively small, but provides special advantages for developmental investigation. For this reason therefore we will endeavour to overcome the difficulty that the cutting of the ripe seed presents. It is advisable first of all to prepare a median longitudinal section through the seed, as we want to know what the object looks like, the development of which we are about to study. This section presents no insuperable difficulty, if we have fresh seeds at our disposal, in preparing between the fingers. It is still easier if we place the seed between two flat pieces of cork, and draw the razor between them. Or the seed can be fastened with gum in the desired position between two pieces of soft lime or poplar wood, and after it is dry the sections made through wood and seed at the same time. Or the seed can be embedded in a drop of gum, to which a little glycerine has been added, at the end of a piece of elder pith, and after drying, gum and seed can be cut at the same time.

The sections, in whichever way prepared, should be examined in glycerine, as water makes the embyro swell, and come out of the testa. The embyro (Fig. 111, *A*), fills up the entire seed; it is bent at its mid-length, so that the **cotyledons** (*c*) lie alongside the hypo-

cotyledonary axis, or **hypocotyl** (*h*) *i.e.* are **incumbent.** (Compare the figure.) This disposition is characteristic of several tribes of the Cruciferæ, by some systematists collected into the section Notorhizeæ, and may be represented by the sign ||O Another characteristic method of folding of the embryo in Cruciferæ, is where the hypocotyl is folded over and applied to the edges of the cotyledons. This is called accumbent, and may be expressed by the sign ⊐O. They may likewise be represented by CCH and ꝯH respectively. If the section is delicate and has cut the seed perfectly in the centre (as in the adjoining Fig. *A*), we see at the base of and between the cotyledons the small growing apex of the stem, the **plumule**, and can also see, at the lower end of the hypocotyl, the axis closed by the **radicle** covered by a **root-cap** only a few cells thick. There is no endosperm to be seen; the embryo is immediately surrounded by the skin of the seed—the **testa,** and is therefore said to be **exalbuminous.** If we use a somewhat higher magnifying power, we can determine that this testa (Fig. 111, *B*) consists of three layers of cells. The innermost layer (*a*) is composed of cells with comparatively little thickened and almost colourless walls, and with granular contents. Addition of iodine shows us that these grains are coloured yellow-brown, and are therefore **aleurone.** Outwardly follows a second layer (*c*), the cell-walls of which are coloured deep brown, and on the inner side are very strongly thickened. The outermost layer of cells appears in concentrated glycerine as a colourless, apparently homogeneous membrane; its cells are strongly flattened, and thickened almost to the obliteration of the lumen. Between the innermost and the second outer layer is often to be distinguished a crushed layer of cells, appearing like a single membrane.—If we examine the testa from the outside, we easily recognise the contour of the polygonal cells of the external tabular layer. These cells have

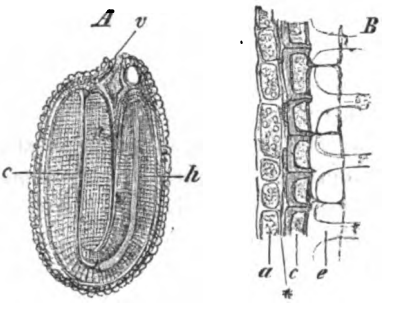


FIG. 111.—*Capsella Bursa-pastoris.* *A*, longitudinal section through a ripe seed; *h*, hypocotyl: *c*, cotyledons; *v*, fibro-vasal bundle of the funiculus (× 26). *B*, part of a longtitudinal section through the testa, after the action of water; *e*, the swollen epidermis; *c*, the brown strongly thickened layer; * the crushed layer of cells; *a*, the aleurone layer (× 240).

their more internal portions partly separated by intercellular spaces full of air. In the middle of each cell is to be distinguished a weakly defined more strongly refractive portion. The walls of the next inner layer of cells are brown, strongly thickened, the cells themselves only a little smaller than in the outer layer. Considerably smaller, on the other hand, and weakly thickened, are the cells of the third, aleurone-containing, layer.—If we now allow water to run into the section from the edge of the cover-glass, we see, in the cross-section, the cells of the outer layer rapidly swell; each bulges strongly outwards; at their centre a highly refractive column is noticeable. The lumen is now hardly distinguishable; the entire cell is filled by the thickening layers of the wall, and in all cases the outer thickening layers are weakly, the internal strongly refractive. These internal thickening layers form the striking central **columella**, which now shows up very strongly on the surface of the seed, while at the same time the intercellular spaces between the cells disappear. The swelling walls usually show clear lamination. With further addition of water, the cuticle of the cells is ruptured, and the outer thickening layers come out, diffusing in the surrounding water as invisible mucilage. The refractive columella remains behind, marking the centre of each cell (Fig. 111, *B*, at *e*). It has increased not inconsiderably in size; at its apex can be seen the relics of the dissolved thickening layers. In the same way the lateral middle-lamellæ of the cells remain, and, as they do not swell, show now a much less height than the columellæ. All this can be seen in our Fig. 111, *B*, which represents the testa after the action of water. These phenomena of swelling can be observed more quickly if the section is first examined in alcohol, and then water run in. This mucilagination of the thickening layers of the outer cells of seeds and mericarps* is a comparatively common phenomenon, which facilitates the sticking of the seed to foreign objects, and therefore serves in their transport and dissemination, and on the other hand has as a result, the firm retention of water on the surface of the seed.

As the section-cutting of ripe seeds presents some difficulty, we can, so far as informing ourselves about the position and structure of the embryo is concerned, prepare the sections from seeds which

* *Mericarps*, the segments of fruits which, like the Pelargonium (Geranium), Cranes' Bills, and Parsley Worts, do not dehisce to let out their seeds, but split up bodily into seed-containing segments. Hence these fruits are called *schizocarps*—splitting fruits. [Ed.]

are not quite ripe, and far softer, and only study the testa upon fully ripe seeds.—Now let us go back to younger stages, and at first lay the entire ovule in potash. These ovules can be best obtained by halving the seed-vessel in its entire length, and then removing the ovules from each half with the scalpel. The ovules, almost to the fully ripe stage, can be made so far transparent that we can inform ourselves sufficiently as to the position of the embryo. The embryo goes a beautiful green in potash, which arises from the swelling of the starch-grains, so that the chlorophyll-grains become visible. Proceeding to progressively younger ovules, we see that the embryo (and at first especially its cotyledons), becomes continually shorter. It continually withdraws further and further from the lower, outward-bent, half of the embryo sac. Ovules from fruits which, without stalk, measure $\frac{1}{5}$ inch in height, show the embryo as a small body of cordate form. The two divaricating anterior protuberances are the rudiments of the cotyledons. If we trace back the various stages of development of the embryo, we can at the same time determine that endosperm is formed only at the two ends of the embryo-sac, and appears especially at the chalazal end as a small green-coloured mass of tissue. The latter is not reached and absorbed till the seeds are well-nigh ripe. We can also see that the testa proceeds from the two layers of cells of the outer integument, and the inner cell-layer of the inner integument. This latter layer is easily distinguished by its richness in contents. The one or two layers of cells lying between this innermost layer and the outer integument are gradually pressed back and crushed, so that they ultimately form only the compound membrane lying between the second and third layer of the testa. If unripe seeds of various ages are laid upon an object-slide in a drop of glycerine, and covered with a cover-glass, and then subjected to carefully controlled pressure with the handle of the needle-holder, the ovules will burst, and the often uninjured embryo will come out. This process is effective with embryos from a very young state up to those that are well-nigh fully formed. —In order to inform ourselves as to the structure of the egg-apparatus in the ovule at the receptive period, we must have recourse to alcohol-material, which we make transparent to the desired degree by careful addition of potash. We can thus state the presence of two synergidæ, and an oosphere, in the egg-apparatus, while the antipodal cells are very difficult to see. The structure of the ovule is easy to follow fresh,

in pure water, or with a trace of potash added to make it still more transparent. The ovule is **campylotropous**, *i.e.*, its nucellus and embryo-sac, as we have already seen in later stages, are curved. The outer integument consists of two layers, the inner in its upper part has two, further in has three layers. The nucellus at this stage is already absorbed, so that the embryo-sac is directly in contact with the inner integument. The funiculus is pretty long; it is traversed by a fibro-vasal bundle, which ends in the chalaza, and is still visible even in the ripe seed (Fig. 111, *A*, *r*) Very beautiful, in the next older stages, especially after the addition of potash, are the views one gets of the rudimentary embryo. We can see that the fertilized oosphere [oospore] grows into a thread-like pro-embryo, about six cells long; the uppermost cell, *i.e.*, that most removed from the micropyle, rounds off into a **head-cell**, the **embryo-cell**, the other cells collectively form the **suspensor**, while the lowermost cell of this embryo-bearer or suspensor, the attaching-cell, swells at the same time bladder-like, absorbs the entire nucellar tissue up to the integument, and forms the bladder which we find at this place even in the ripe state (comp. Fig. 111, *A*). This swollen cell may serve to help on the absorption of nutriment for the embryo. The tissue of the chalaza swells considerably at the same time, and the cell-contents become dark-coloured. Soon we see there the green endosperm-cells, which in smaller number surround the rudimentary embryo at the micropylar end also. In such preparations we can determine that the swollen globular embryo-cell is already separated from the suspensor by a partition wall, and soon after is divided by a longtitudinal wall, at right angles to which follows a second longtitudinal wall, and then at its mid height a cross-wall. The globular embryo thus appears divided into octant-cells, in which are subsequently formed periclinal and anticlinal walls. The globular embryo increases in size and in number of constituent cells, flattens somewhat, and then from its anterior end the cotyledons grow out. These at first are in contact at their base, and then subsequently the growing point or plumule of the stem bulges out between them. All of these stages can likewise be followed in fresh material, and *in situ*, by laying the ovules in glycerine, covering with a cover-glass, and then carefully heating over a spirit or gas-flame. The ovule is thus made transparent, and the embryo clearly visible.

For the study of the **monocotyledonous embryo** we select the common Water Plantain, *Alisma Plantago*.[2] This object is, in fact,

highly suited to this kind of investigation, and is therefore very commonly used for it. First of all we will make ourselves familiar with the fully-developed state. The flower of *Alisma Plantago* contains numerous monocarpellary pistils; it is **apocarpous**, much resembling in this respect the Ranunculaceæ. From each flower therefore proceed numerous fruits, which are closely pressed together in a single whorl into a collective fruit, a dry **etærio** of triangular outline. Each individual ripe carpel [**achene**] is strongly flattened laterally, somewhat thicker above, obovate in profile, with a median dorsal groove. At about mid-height of each achene, on its internal edge, is a short thread-like outgrowth, representing the withered style which, therefore, is ventral. For further investigation we select an almost ripe etærio, place a single achene between the two halves of a split cork, and draw the razor between these two halves, we shall thus, without trouble, obtain tolerably median longitudinal sections; while cutting between the fingers presents difficulties, as the testa is so hard. At the same time we will prepare some cross-sections in the ordinary way, between two pieces of cork. The longitudinal sections we examine in water, to which we add a little potash. For the cross-sections pure water suffices. For the study of the testa upon the longitudinal sections the air must be driven out; for this we can either lay the sections for a short time in alcohol, or else place them under the air-pump. We also lay some longitudinal sections in carbolic acid, and in this way obtain figures which supplement the

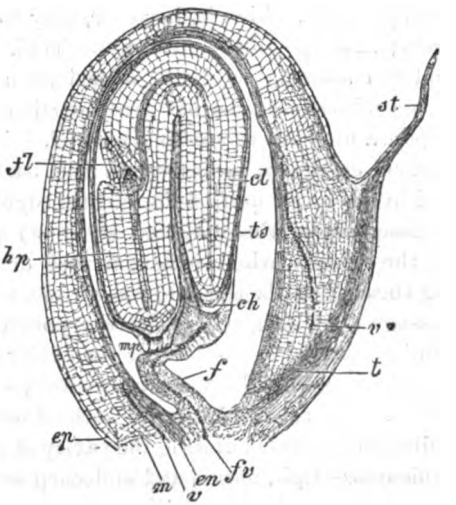


FIG. 112.—*Alisma Plantago.* Median longitudinal section through a ripe achene. *ep*, epicarp (epidermis); *m*, mesocarp; *en*, endocarp of the wall (pericarp) of the fruit; *v*, a fibrovasal bundle in this; *v**, the end of the fibro-vasal bundle; *st*, the withered style; *t*, the pollen-passage; *f*, funiculus of the seed, with its fibro-vasal bundle *fv*; *mp*, micropyle; *ch*, chalazal end; *ts*, the testa; *hp*, hypocotyl of the embryo; *fl*, first leaf; *cl*, cotyledon (× 23).

others in the most satisfactory way. The longitudinal section, if correctly cut, presents the appearance of the adjoining Fig., 112. We have first a comparatively thick wall to the fruit, the **pericarp,** which in its surface is covered by the epidermis (*ep*). This is, as our median longitudinal section teaches us, a pretty sharply delimited part of the pericarp, and is therefore distinguished as **epicarp.** To the epidermis follows a parenchymatous tissue of approximately isodiametric cells, strongly thickened, filled with air, and without intercellular spaces; this forms the **mesocarp** (*m*). Internally follow several layers of elongated sclerenchymatous elements, and represent the **endocarp** (*en*). A perfectly median longitudinal section cuts, at the back of the testa, a mucilage-passage adjoining the epidermis, which is, however, only to be seen well in an unripe testa; in a ripe one, on the other hand, it appears almost empty, and can scarcely be distinguished from the neighbouring tissue. Longitudinal sections not truly median may, on the other hand, expose a fibro-vasal bundle (*v*), which, lying close to the sclerenchymatous endocarp, passes up at the back of the fruit, in order to end in the lower half of the ventral edge (at *v**). Under the place of insertion of the withered style (*st*) projects the ventral edge of the epicarp, which is here formed of elongated cells. Adjoining these towards the interior we see, in favourable cases, an air-passage (*t*) which, continuing the pollen route through the style, can be followed to the base of the cavity of the ovary. This is the passage by which the pollen-tubes arrive at the micropyle. As the ovules turn their micropyles to the dorsal edge of the ovary, the pollen-tubes, after entering the cavity of the ovary, grow round the funiculus.—Epi-, meso-, and endocarp are even easier to recognise in cross than in longitudinal sections; and the furrow in the middle line of the back is now specially noticeable. The seed almost completely fills up the cavity of the ovary, as the median longitudinal section through the fruit proved, and is fixed in the central position at the base of the cavity of the ovary by a tolerably long, bent funiculus (*f*). A fibro-vasal bundle enters into this funiculus. The seed is **campylotropous,** and completely filled by the embryo. As testa (*ts*) only a thin skin is present, consisting of two clearly distinguishable layers of cells. Between the two we see here and there also a third crushed layer, which comes out more clearly after swelling in potash. The inner layer of cells of the testa is strongly thickened on the inner side. The micropyle (*mp*) is very prominent on the seed. The root-end of the embryo

lies directly against this on the inside. This root-end is somewhat swollen, and projects in the middle in the form of a papilla. If the section has cut the embryo quite centrally, we see that the papilliform projection is formed of two layers of root-cap, which at the edges pass over into the epidermis. At mid-height of the seed is to be seen upon the embryo an outward-turned narrow notch, in which lies the growing apex of the stem [plumule]. This growing apex is enclosed in the cotyledonary sheath. Upon the apex arises a median, external (see figure, towards the left-hand) rudimentary leaf, which completely fills up the notch. The part found between this growing apex and the end of the root is the hypocotyl. It is covered by the epidermis, shows three layers of **cortical cells**, regularly arranged into a cylindrical cover, and a median string of elongated cells which runs from the root-apex towards the growing point of the stem. These cortical layers have only one layer of common **initial cells** at the apex. Over these runs the **dermatogen**, from which two layers of root-cap appear segmented. The central string, the **plerome**, is terminated by its own initials. The hypocotyl is prolonged into a cotyledon. This is bent, in proportion to the form of the ovarian cavity, tapers gently to its apex, and ends at the chalazal end of the seed. The cotyledon also consists of a cylinder of regularly arranged layers of cells, and is traversed by a central string of elongated cells. This string bends in under the growing apex of the stem, and joins with that of the hypocotyl (see figure). The cell-rows of the cortex as well pass over from the hypocotyl into the cotyledon with a gentle bend. The cotyledon shows in its lower part, like the hypocotyl, three, higher up, corresponding with its diminution, two, and finally only one layer of cortical cells. The central string ends at some little distance from the apex of the cotyledon. In the ripe seed there is not a trace of endosperm. This seed likewise is ex-albuminous. The embyro itself has all its cells densely filled with starch.—The cross-section of the seed shows nothing new. It presents two cross-sections of the embryo at the same time, separated by a narrow strip of tissue which passes over into the inner cell-layer of the testa. The structure of the testa is more clear than in longitudinal sections. The cross-sections of the embyro show the concentric arrangement of the cortical layers very beautifully.

The two angiospermous plants investigated by us offer us truly typical but extreme examples of embyro formation in dicotyledons and monocotyledons respectively; types which are far from

exhausting the whole diversity of cases which have been investigated. Thus, amongst dicotyledons are examples of embryos which have only one cotyledon (*Carum Bulbocastanum*, the Pignut, *Ranunculus Ficaria*, the Pilewort); and amongst monocotyledons those in which the cotyledon arises laterally from the growing apex of the stem (Dioscoreaceæ, Commelynaceæ).[3]

The two cases studied above are both examples of **exalbuminous seeds**, *i.e.*, of seeds in which the endosperm formed in the embryo-sac is completely, or almost completely, absorbed into the embryo during its early growth, and stored up mostly in the interior of the cells of the cotyledons. An example of the other primary, but less common, type of seed, known to systematists as the **albuminous seed**, *i.e.*, one in which the endosperm formed in the embryo sac is not absorbed by the embryo until the period known as germination, has been already in part studied by us in Chapter II., especially in the case of *Triticum* as a Monocotyledon, and *Ricinus communis*, the Castor Oil, as a Dicotyledon. It is advisable for the student to complete his study of the structure of seeds by a careful study of the ripe seed of this latter plant, first removing the testa, as it is too hard for satisfactory section cutting. The embryo is straight; the two large, delicate, veined, cotyledons lie on either side of a narrow central cavity, which nearly separates the seed into two halves; the radicle projects towards the curious, wart-like **micropylar aril** which will have been noticed at one end of the testa. The information given in the above chapter will enable the student to fill in the details of the structure of the seed.

On account of its special interest, we will, however, make a further study of the **grain of wheat**, the fruit of *Triticum vulgare*. The ovary of the flower of the wheat-plant is superior, or free, and contains a single ovule. This latter, in its development, completely fills the cavity of the ovary, the pericarp adhering to the testa, as is shown in Fig. 11, p. 19, and both being strained and flattened, so that the constituent cells collapse. We may investigate either softened, or, what is even better, just ripe grains. If we take softened grains, we must take care that the grain is only just soft enough to make it suitable to cut. The ripe grain of wheat shows in the middle line of its inner side a deep furrow, corresponding with the ventral suture of the ovary. At the base of the opposite side is the embryo, recognisable as a slightly elliptic protuberance ending conically below. The flattened top of the

grain is crowned with hairs, between which is the filiform remnant of the style. The grain of wheat is not a naked seed, but a one-seeded, dry, indehiscent fruit, a **caryopsis**.—Take first of all cross-sections of the grain at about mid-height, and examine them in water or in glycerine, and later on with the addition of a little potash. Leaving the furrow out of the question for the present, we find that the exterior is constituted of one or several layers of thickened pitted cells, the walls of which are highly refractive and yellowish, and colour a deep yellow in potash. The outermost layer of these cells is the epidermis (Fig. 112A, *A ep.*), the succeeding layers (*e*) belong to the inner tissue of the wall of the fruit, or pericarp, and the innermost layers are for the most part obliterated. To this outer tissue succeeds a layer of tangentially elongated, straight, or more or less curved, cells (*chl*), distinguished by numerous narrow, cross-set pits. Now and again in the inner side of this pitted layer we see tube-like cells, which indicate the inner epidermis of the pericarp. This is all that remains of the pericarp. The tissue next following appertains to the seed. It is separated from the pericarp by more or less numerous air spaces. The **testa** shows first a thin, apparently homogeneous, colourless skin, arising from a layer of cells with obliterated cavities; to this follows a similar narrow layer, the scarcely recognisable cavities of which have brown contents. These two layers are represented in *ti*, in Fig. 112A, *A*; together they constitute the testa; the outlines of their cells are recognisable upon tangential sections. All the elements of pericarp and testa are, so far as they possess cavities, filled with air. To the testa succeeds a relatively thick, strongly refractive, white skin (*n*), which owes its origin to the outermost layer of the tissue of the nucellus. In ordinary seeds this would be known as the **tegmen**. The original cavities of the cells in this are indicated by narrow, granular, tangential striæ. To this layer follows the layer of radially elongated aleurone-containing **endosperm cells** (*al*), which we have already studied in Chap. II; and next to these come the cells of the inner endosperm, filled with starch. The wall of the furrow shows the mesocarp much increased in quantity, and the layers progressively of larger cells; while in the middle line the elements are again small, thin-walled, without interstices, and have a median, weakly developed vascular bundle. The layer *chl* is thicker, and contains chlorophyll and large air-cavities; the outer nucellar layer thickens into a cushion of tissue, behind which the aleurone layer is often wanting.

This increased development of the outer tissues accompanies a deeper infolding of the endosperm than of the testa and pericarp, and a sharp limit between the tissue of the seed and of the fruit is not recognisable in the furrow. A tangential section taken from the surface of the grain shows us that the epidermis and adjoining tissue of the wall of the fruit consists of longitudinally elongated cells, and the tissue of the pitted layer, on the other hand, of horizontally elongated cells, and that the outer layers therefore consist of cells set at right angles. The best impression of these layers is

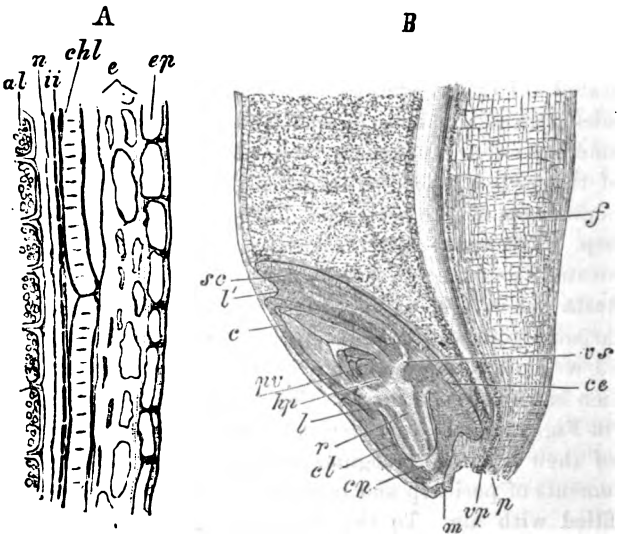


FIG. 112A. Grain of *Triticum vulgare*, the wheat.—*A*, cross-section through the pericarp and testa. Of these, *ep* is the epicarp, *e*, outer layers, and *chl*, chlorophyll layer, appertaining to the interior tissue, or mesocarp; *ii*, remnants of the ovular integument; *n*, the outermost thickened layer of the nucellus; these together constituting the testa; *al*, the aleurone-layer of the endosperm (× 240). *B*, median longitudinal section through the lower part of a ripe fruit. At the bottom of this to the left is the embryo with the scutellum, *sc*; *l'*, the ligule of the scutellum; *vs*, its vascular bundle; *ce*, its "cylindrical epithelium"; *c*, the sheath of the cotyledons; *pv*, the growing apex of the stem; *hp*, the hypocotyl; *l*, the ligule upon it; *r*, the radicle; *cp*, the root-cap of the radicle; *cl*, the root-sheath (coleorhiza); *m*, place of exit of the radicle, corresponding with the micropyle of the ovule; *p*, the funicle; *vp*, vascular bundle in the funicle; *f*, lateral wall of the furrow (× 14).

obtained if the sections are laid for a couple of hours in chloral hydrate, and afterwards examined in the same fluid.

Let us now endeavour to obtain a tolerably median longitudinal section through the ripe grain, for which purpose we must not, however, use air-dry, but softened, or even better, grains which are just ripened. The embryo will show specially well in sections

examined in carbolic acid, or treated with potash and examined in glycerine. We will first examine the section with a low power, and afterwards work over it carefully with a high power for the purpose of detailed study. We begin with the **embryo**. This is situated obliquely, at the base of the body of the endosperm (Fig. 112A, *B*), and is in contact with it by means of the **scutellum** (*sc.*). The scutellum appears in longitudinal section as a flat structure, which at both upper and under termination ends free in a blunt projection. Below the upper end a short excrescence arises from the scutellum (*cf.* figure), which is to be looked upon as a ligular structure (*l*). Bounding the scutellum in the upper half of the embryo is the sheath-like **cotyledon** (*c*). This sheathing cotyledon surrounds many rudiments of leaves, which decrease in size inwards. The largest of these leaf-rudiments stands in the middle line towards the exterior. Between the youngest (innermost) of the leaf-rudiments lies the growing apex, from this point of view appearing relatively narrow and steep (*pv.*). Together with the leaf-rudiments this forms the rudimentary bud, the **plumule**. The plumule and the cotyledon are borne upon the stem, or **hypocotyl** (*hp*). Upon its outer side this produces a small free **ligule** (*l*). The hypocotyl is prolonged below into the **radicle** (*r*), which is dissected somewhat obliquely forwards. Even with low magnification this shows the central **plerome**, closed cone-wise towards the apex, and surrounded by the **periblem** and **dermatogen**. At the apex the periblem and dermatogen come together into a single layer of cells (compare with this Fig. 69, p. 184). Inside the plerome-cylinder the rudiments of the first vessels can be very easily seen, and traced right up to its apex. The **root-cap** (*cp*) lies over the apex of the root as a transparent cover. The whole of this rudimentary root lies in a closed sheath, the **coleorhiza** (*cl*), and is sharply limited towards it by a clear line, which indicates the thickened walls of its dermatogen cells. This clear line is lost over the root-apex, between the body of the root and the root-cap. At its base this root-sheath passes over into the tissue of the hypocotyledonary axis. At its part which ensheaths the root-apex the coleorhiza is swollen into a wart-like, clearly distinguishable projection (compare the figure). A string of elongated cells (*vs*) can be traced from the hypocotyl into the scutellum; the epidermal cells (*ce*) on the outer surface of the scutellum are distinguished by their considerable radial elongation. The scutellum is a

sheath-like outgrowth from the base of the cotyledon, and must therefore be looked upon as a part of it. It remains in the seed during germination, and serves as a suctorial organ. The absorption of the food-materials takes place by means of the cylindrical epidermal cells (epithelium), and continues until the whole of the reserve food-materials of the endosperm are exhausted.

The embryo lies in immediate contact with the **testa**, while the **pericarp** is here somewhat thicker, but more loosely constructed. Under the wart-like apex of the coleorhiza, *i.e.*, as the place from which, in germination, the radicle will project, there a still further reduction and a depression (*m*) is visible. The fruit has a very short stalk (*p*), and we can see the vascular bundle (*vp*), which enters here and passes into the funiculus of the seed, which is fused, however, with the pericarp, and the vascular bundle is hardly recognisable. Further inwards, however, can be seen a much more noticeable string of grey-brown elongated, slightly pitted cells, which we have already noticed in the cross-section. The vascular bundle itself is embedded in delicate walled, colourless, slightly elongated cells. Further inwards are the layers of nucellar cells, with fairly thick, white walls, and then comes the aleurone layer of the endosperm. This is readily separable from the nucellar cells, so that an air space is often present at this part. Towards the embryo the endosperm is not bounded by an aleurone layer, but by a fairly thick layer of swollen cell-walls, which represent endosperm cells which have been crushed back by the enlarging embryo.

The ripe grain of wheat germinates very easily, and we will therefore make use of it in order to study the early stages of germination. It will suffice to embed ripe grains in moist sawdust; it is even enough to stand ripe ears for a few days with their lower ends in a glass of water. The pericarp of the grain is first of all broken through at its weakest part (*m*), that which corresponds with the micropyle of the seed: the coleorhiza protrudes, and out of its tip the rapidly lengthening radicle soon projects. The coleorhiza surrounds the base of the radicle like a sheath. Above this point the lowermost pair of lateral roots subsequently arise, each likewise surrounded at its base by a root-sheath, or coleorhiza. The whole grain swells considerably, and ruptures the pericarpic covering more or less completely. If this is lifted off, we can easily see, with the lens, the ligule between the bases of the two lateral roots. The cotyledonary sheath

elongates, and assumes a greenish colour. After it has attained about fifty-fold its original length, it is broken through at its apex by the first bright green foliage leaf. Considerably later than the first, or lower, pair of lateral roots is produced a second, upper pair. The original distance apart of the structures in the neighbourhood of the lateral roots remain unchanged, and shows that the hypocotyl undergoes no considerable elongation. The lateral roots soon equal in development the principal roots, a tap-root is therefore not produced.—From a seedling which has already produced all its rudimentary roots, we now cut off all the elongated parts, and prepare a median longitudinal section through the fruit. We can now easily see that the growing point remains pretty much in its old position, having only developed a number of new leaf-rudiments. The scutellum has in general not increased in size, excepting as to its cylindrical epithelium, the cells of which have elongated still more, and more or less completely isolated from one another laterally, so as to resemble hairs; they have rich protoplasmic contents.—It is of great interest for us now to take a little of the endosperm tissue, diffuse it in a drop of water, and examine it with a high power. Amongst the more or less numerous starch-grains which are still unchanged will appear others which have become corroded by the action of diastatic ferments which have been produced in the course of germination. Such grains appear peculiarly changed. Partly still white, of their original density, and without clear lamination, they are in other places transparent, sharply laminate, and with the concentric layers traversed by more or less dense radial striæ. Many grains appear as if reduced thereby to vermiform particles, and finally such grains are completely dissolved.

NOTES TO CHAPTER XXX.

[1] Compare Hanstein, *Bot. Abhandl.*, Bd. I., Heft. i., p. 5. Westermaier, *Flora* 1876, p. 483. Famintzin, *Mém. de l'Acad. Imp. d. Sc. d. St. Petersb.*, VII. Sér., Tom. XXVI., N. 10. Kny, *Bot. Wandtafeln*, Heft i., p. 20. A collection of all embryological literature in Goebel, *Vergl. Entwicklungsgeschichte*, in Schenk's *Handb. d. Bot.*, Bd. III., p. 165 *et seq.*

[2] Hanstein, as above, p. 33; Famintzin, as above, p. 4.

[3] The literatue is in Goebel, as above, p. 169 *et seq.*

CHAPTER XXXI.

THE FRUIT OF ANGIOSPERMS.

Material Wanted.

Ripe plum or cherry, and apple. Fresh.

Ripe orange, and young orange ovaries of various ages, from the full-blown flower. Fresh. These can be obtained all the year round.

One of the simplest possible cases, namely the formation of the capsule from the inferior ovary in Orchideæ, we have already come to know; likewise an even simpler case in the achene of *Alisma plantago;* in the following pages we will turn our attention to some more complicated cases.

A ripe plum, *Prunus domestica*, shows upon its surface a delicate covering of **wax**, the so-called "bloom," which in a surface view of the epidermis appears as a finely granular covering. The same view shows the epidermis composed of cells, which, combined into groups, betray clearly their origin from common mother-cells They contain rose-red cell-sap. A delicate cross-section shows us under the epidermis some layers of cells rapidly increasing in size, and further in cells which remain uniformly large. These are rounded off from one another, but form however only small intercellular spaces. They contain very small, scattered, yellowish-green chlorophyll-grains, a thin peripheral layer of protoplasm, a nucleus, and for the rest colourless cell-sap. This parenchymatous tissue is traversed by numerous branching fibro-vasal bundles. Towards the stone the parenchymatous tissue becomes smaller celled, radially elongated. The **stone** itself, which, in order not to break the razor, must be cut with the greatest possible care on a surface previously prepared with a strong pocket-knife, consists of very strongly thickened and lignified elements, the walls of which are traversed by beautifully branched canals. The history of its development shows that the stone belongs to the wall of the ovary, the **pericarp**; and that the epidermis of the plum, the epi-

carp, arises from the epidermis of the ovary; that the **flesh** of the fruit, the **mesocarp**, arises from the tissue of the ovary underlying the epidermis; and the stone, or **endocarp**, from its inner tissue. The entire tissue of the plum, including the shell of the stone, owes its origin therefore to the wall of the ovary. Surrounded by the stone is the seed, consisting of the embryo, a delicate testa, and a remnant of the endosperm remaining between the embryo and the testa. If we cut it across we can easily distinguish the two cotyledons, lying flat together. A median longitudinal section of the seed shows us at its base, between the two cotyledons, the hypocotyl of the embryo, with its radicle projected into the pointed micropylar end of the seed, and between the base of the two cotyledons the bud or plumule. The embryo, during its increase in size, has absorbed the entire tissue of the ovule, up to the very thin testa, from which still arises, quite close to the micropyle, the withered funiculus. Delicate cross-sections through the seed show us the testa composed of collapsed layers of cells, and covered on its outer side with rounded cells, thickened either only or chiefly upon the sides, bulging outwards, and standing either singly or in groups. Between the testa and the cotyledons is a more or less thick layer of endosperm, here and there reduced to a single layer of cells, or entirely absorbed. Surface sections of the testa show us that the thickened projecting elements are single cells or groups of cells of the testa. These have been thickened while their neighbours remain unthickened; and as these latter collapse the former project. The pits, abundant upon the side walls, give to these cells an elegant appearance. Where two thickened cells are in contact, the pits come together. The history of its development shows that the testa arises from one integument of the ovule. Two ovules are present in the ovary, of which only one is developed further. Such a fruit as the plum is known as a **drupe**.

This description of the plum will, apart from unimportant differences, serve likewise for the cherry, which therefore can be investigated in the place of the former. This enlarges the opportunity of examination in the fresh state, since cherries or plums can be obtained, more or less ripe, from April, or even earlier, till October, inclusive.

We will now make ourselves acquainted with the microscopical structure of an apple (*Pyrus Malus*). The apple, like the plum and cherry, belongs to the **fleshy indehiscent fruits**; while, how-

ever, the plum or cherry owes its origin to a superior ovary, formed from a single carpellary leaf, the apple arises from an inferior, five-celled ovary, composed of five carpellary leaves. However, having regard to the relations which the nearly allied roses offer, we can assume that the five-celled ovary here is immersed in a hollowed flower-stalk, a so-called *hypanthium* or **receptacular tube,** and is adnate to this. Many reasons concur to render this conclusion highly probable. To describe the apple or the hip [fruit of the rose] as a pseudo-fruit is at any rate incorrect, since the structure producing the apple differs in no way from the inferior ovary of many other plants. The apple is crowned at its top by five more or less completely shrivelled sepals, and also by the withered relics of the rest of the floral parts. Surface sections show the epidermis of the apple to be composed of comparatively small polygonal cells, upon which grouping, as a result of development, can still be recognised. The walls of the cells are pretty strongly thickened, their cell-sap either colourless or rose-coloured. The surface of the epidermis is covered with a finely granular covering of wax. The small prominences, which are readily visible on the surface of the apple with the lens, are occupied in their centre by a stoma. The tissue under such a stoma is often dead, or else the epidermis is here ruptured, and the wound closed with cork. Thin cross-sections show us that the epidermis is strongly thickened on its outer side. Below it lie several layers of tangentially elongated cells, with tolerably thick walls, which, passing inwards, gradually become larger and thinner-walled, and at the same time chlorophyll-containing. No sharp limit between epicarp and mesocarp is therefore present. The chlorophyll-grains are filled with starch; their colour disappears towards the interior of the apple, they at the same time become less numerous; at length, at a certain depth, the large bladdery cells of the mesocarp contain, besides the delicate peripheral layer of protoplasm and nucleus, for the most part only colourless cell-sap; the intercellular spaces are here filled with air. Fibro-vasal bundles are scattered in the entire tissue.—The five cells, forming the "core," are covered each by a smooth, hard, cartilaginous membrane, the endocarp. This corresponds with the shell of the plum-stone. It consists of several layers of sclerenchyma-fibres, thickened almost to the obliteration of their cavity, and the thickening layers of which are pierced by fine pores. Surface sections show that these sclerenchyma-fibres slope irregularly, often are bent,

and in the different layers have oppositely inclined courses. The five cells often separate in the middle, forming a central cavity, into which the individual cells then usually open. At the base of each cell are inserted two ovules, of which both, or only one, produce seeds, or of which commonly neither develops further. The seed is almost filled by an embryo of the same structure as the plum or the cherry. The brown testa, on the other hand, is much thicker than in the last-named plants. In cross-section it shows an epidermis, the cells of which outwardly are strongly thickened, the external layers colourless, and capable of strongly swelling, the inner brown-coloured and not swelling. In sections laid in water the swelling layers, increasing in volume, at length break through the cuticle, and project outwards like papillæ. It is these which make the wet seeds slippery. The thick tissue underlying the epidermis appears in cross-section to be formed of polygonal cells, rounded at the angles, brown, and pretty strongly thickened, to which succeeds a layer of cells only about one-third so thick, tangentially elongated, but less strongly thickened. These border on a shining white, thick membrane. This last arises from the strongly thickened outer walls of the outermost layer of the nucellus; the entire remainder of the testa comes from the outer integument of the ovule. The inner integument of the ovule is very early absorbed. The nucellar cells, the thickening layers of which appertain to the testa, are mostly collapsed, as also are the rest of the cells of the nucellus which are present. To this collapsed layer of tissue succeeds a thin layer of endosperm, which is also absorbed here and there, and, so far as it is present, sheathes the embryo. The endosperm-cells are densely filled with aleurone-grains. As successive surface sections show, the epidermis consists of cells which are comparatively but little elongated, the inner thickening layers of which are porous. The tissue following the epidermis, which in cross-section appears isodiametric, shows now to be elongated in longitudinal direction and provided with obliquely mounting cleft-like pits. The radially elongated inner elements of the testa are arranged at right angles to the preceding.

The cross section through a ripe orange, *Citrus vulgaris*, [or *C. aurantium*,][1] shows externally the part designated **rind**, and internally the cells filled with the orange-red **flesh**, the number of which cells is indefinite, and varies from 6 to 12. The cells are separated laterally by thin partition-walls, which combine into a

central column of tissue. If you wish to apply the customary designations of the parts of a fruit to the structure of this, we can speak of the outer rind as epicarp,* the orange-red flesh as mesocarp, the inner column of tissue and the partition-walls as endocarp. We enter now into a microscopical investigation of the individual parts. In delicate cross-sections through the rind we see most externally a small-celled epidermis, to which adjoins a tissue of gradually enlarging cells. The epidermis and adjoining tissue contain orange-red chromatophores, which further inwards disappear. Intercellular spaces filled with air appear here between the cells, and gradually enlarge, since the tissue itself acquires the character of a looser spongy parenchyma. The elements of this latter are extended in the tangential direction. The rind is traversed by fibro-vasal bundles, which the cross-section lays bare chiefly in the direction of their long axis, and which branch towards the periphery. Adjoining the epidermis are the large glands of ethereal oil, visible to the naked eye. They show throughout the structure known to us from *Ruta*, and allow the inner investment of delicate cells to be readily distinguished. The fruit, observed macroscopically from the outside, shows the oil-glands as darker spots, the tissue separating them as a brighter net-work. —A delicate surface-section of the outer side shows us first the small polygonal epidermal cells. Those lying over the oil-glands are distinguished by a want of the orange-red chromatophores; they contain in place of them colourless globules of various sizes. Scattered in the epidermis are the stomata, devoid of protoplasm, and closed on the inner side. The next deeper sections give instructive views of the oil-glands and terminations between them of the fibro-vasal bundles. Still deeper sections show the spongy tissue of elongated sac-like cells. Adjoining the loculi of the ovary the cells of the rind become still longer, fibrous, in part strongly thickened and then provided with narrow, obliquely mounting pits. The partitions between the loculi are constructed in the same way: in the interior, of spongy parenchyma; outwardly, of fibrous, in part thickened tissue. The spongy elements, found outside the loculi as well as in the interior of the partitions, easily fall out of union. The fibrous elements, on the other hand, appear pretty firmly connected together. The best view of these latter is obtained in surface view. We separate the contents of the loculi from one another in the customary way; the spongy tissue sur-

* This view is not generally held in England. [Ed.]

rounding the loculi is thus torn, the fibrous layer remains, however, as a delicate white sheath around the flesh of the fruit. If we now spread out such a sheath and examine it with strong magnification, we see it constructed of several layers of fibres, running parallel to the surface of the loculus and at right angles to its long axis. Between unthickened fibres are scattered others of similar form, thickened and pitted.—The flesh of the fruit consists of club-shaped sacs, of which it can be readily identified, even macroscopically, that they all arise from the outer side of the loculus. They are here inserted with a narrow base, and, crowded together, fill up the loculus. The more deeply they extend into the loculus the longer they are; their course is radial, at right angles with the long axis of the loculus. Each individual club shows at its surface to be surrounded by a layer of closely-united elongated fibrous cells, just as we see them in the boundary of the loculus. Interpolated between these cells are single ones, more strongly thickened, and provided with obliquely ascending pits, The interior of the clubs is, however, filled with very large, polygonal, delicate-walled cells, full of sap, in the interior of which are visible spindle-shaped, very narrow, orange-red chromatophores. The central core of tissue, in which the partitions come together, is formed of the same spongy parenchyma as in the internal part of the rind.—In "quartering" an orange we free, as we see, the contents of the loculi, surrounded by the fibrous layer clothing each loculus, which is easily separated from the spongy parenchyma. This fibrous layer can now be very easily separated from the sides of each "quarter," with more difficulty from its outer surface, because here the sacs of the flesh are joined to the fibrous layer. In the flesh the seeds lie embedded in uncertain number. They occupy the inner edge of the sections, turning their place of insertion inwards. In isolating the sections the seeds are separated from the placenta; usually, however, portions of the inner core of tissue, together with the placentæ, cling to the inner edges of the sections.

As the orange trees of botanical gardens, etc., readily provide the required material, in the way of fruit in all stages of ripeness at the same time, we will endeavour to trace out the development of this fruit, stopping, however, at only the most important stages. The cross-section through an ovary taken from a flower shows already a pretty thick wall, having oil-glands in its periphery, and also a strongly developed central core, while the loculi appear

comparatively small. The ovules are inserted in two rows in the inner angles of the loculi, and with their long axis arranged radially outwards. The loculi are clothed with epidermis, which is bounded by two or three layers of a tissue without inter-spaces, while further in the tissue has air-containing intercellular spaces. From the outer surface of each loculus small protuberances already project inwardly; in their formation the internal epidermis and the next succeeding cell-layer take part. The cross-section through a small rudimentary fruit of about $\frac{1}{5}$ inch diameter shows in the place of the small protuberances cylindrical, small-celled emergences, which extend to various depths in the loculus, and have already begun to press in between the rudimentary seeds. Their epidermis is continuous with that of the loculus, while their inner cells pass over into the hypodermal tissue surrounding the loculus. Individual emergences remain at an earlier stage of development, and the cells of their surface grow out into papillæ. The older now the rudimentary fruits we investigate are, so much the longer are the sacs which fill up the enlarging loculi. The loculi, however, at first always remain very small in proportion to the rapidly thickening rind, in the periphery of which the number of oil-glands undergoes corresponding increase. The flesh-sacs now begin to swell into a club in their upper part, their epidermis to elongate in the long direction of the sac, while the inner cells of the sac by successive cross-division remain isodiametric. Moreover a highly refractive, yellowish content distinguishes the inner cells of the sac from its epidermis. A notable elongation parallel to the surface of the loculus is undergone also by the epidermis clothing the loculus, and the layers contiguous to it, which were early distinguished by the want of interstices. All this is already completed in a rudimentary fruit of from $\frac{3}{5}$ to $\frac{4}{5}$ inch diameter, and the important stages of development are therefore cleared up, for the sacs now only need further to enlarge, and to differentiate, in order to attain the stage already known to us in the ripe fruit; from the epidermis of the loculus and the tissue adjoining it, proceed however the fibrous layer surrounding the segments of the fruit; the tissue of the central core, and of the rind, which already contains air, produces the spongy parenchyma; in the periphery of the rind the oil-glands are in course of progressive development, and the layers which now contain chlorophyll will later contain the orange-red chromatophores.

Cross-sections through an ovary taken from a flower, treated

with potash, easily show us ovules[3] in median longitudinal section. The ovules are anatropous; we determine upon them the existence of two thick integuments, of a nucellus, and, in perfectly median sections, also a small embryo-sac. Pollination and fertilization in the orange are separated by about four weeks. To study the processes of fertilization offers difficulties; if we turn, however, to the rudimentary seeds of a fruit about $\frac{1}{8}$ inch thick, we can easily, in longitudinal sections prepared between the fingers, find in the apex of the embryo-sac the still few-celled rudimentary embryo. The nucellus is hollowed in the form of a funnel, and the way by which the pollen-tube has passed into it is marked by small cells rich in contents. Of the inner integument the innermost layer of cells is distinguished by their brown coloration, and the small size of the elements. The inner integument is only a few cells thick, while the outer has considerable thickness. Upon this latter the epidermis begins to fill with finely granular contents, and to thicken on the outer side. If the rudimentary seeds have attained a length of from $\frac{1}{6}$ to $\frac{1}{5}$ inch, a very peculiar phenomenon is to be observed in them. In the immediate proximity of the apex of the embryo-sac,—or, now and again, even at a considerable distance from it,—projecting protuberances show themselves in the hollow of the embryo-sac, which clearly are traceable to luxuriant growth of the surrounding nucellus. In this way in *Citrus*, as in a number of other Angiosperms; **adventitious embryos** are produced, besides the fertilized embryo. Median longitudinal sections through the next older rudimentary seeds show us the same kind of rounded rudimentary embryos, in different stages of development, projecting into the embryo-sac, and with especial frequency at its anterior end. Here and there we can make out that the embryo developed from the oospore has likewise further developed. The formation of endosperm quickly follows, and on longitudinal sections through the next older rudimentary seeds we find the embryo-sac completely filled with endosperm. Into this latter the young embryos are pushed, and some of them soon begin to produce their two cotyledons, and to assume the typical form of the dicotyledonous embryo. The nucellus is absorbed by the embryo-sac up to the outermost layers of cells. Of the outer integument the epidermal cells have elongated considerably in longitudinal direction, and at the same time increased in height. The thickening of their outer walls has gone on very strongly. The other tissue of the outer, as well as

that of the inner integument, have, on the other hand, undergone no important change. As we can make out upon still older rudimentary seeds, the embryos soon begin to be impeded in their development; one or more get the upper hand, and, after all the endosperm has been absorbed, fill up the embryo-sac. The longitudinal section through the ripe seed then shows either one or several embryos pressed together; besides those fully developed are also some incomplete and imperfectly developed. **Polyembryony** in the orange is therefore dependent, not on the presence of several oospheres in the embryo-sac, all capable of fertilization, but on the formation of adventitious embryos. The testa consists of the outer cell-layers of the nucellus, densely filled with contents, and of the two integuments. The limit of these two towards one another is obliterated, while on the other hand the innermost layer of the inner integument is well marked by its brown colour. The epidermis of the outer integument has attained considerable height, and is thickened on its side walls also by newly formed obliquely-pitted thickening layers. The thickening masses laid on outwardly swell with lying in water, and give the seed its slippery mucilaginous surface. The last produced, inner thickening layers increase in volume in their upper parts, and project outwards like papillæ.

NOTES TO CHAPTER XXXI.

[1] Compare Poulsen, *Botaniska Notiser utg. of Nordstedt*, 1877, p. 97, where the other literature is given.

[2] E. Strasburger, *Jan. Zeitsch. f. Naturw.*, Bd. XII., 1878, p. 652.

CHAPTER XXXII.

CELL-DIVISION AND NUCLEAR DIVISION.

Material Wanted.

Young flower-buds of *Tradescantia Virginica*, or allied species, June to August. Fresh.

Flower-buds of various ages of some Liliaceous plant, such as *Fritillaria Persica*, or other species, Tulip, *Lilium*, etc. Fresh. Also the same in absolute alcohol.

Flower-buds, various ages, of a Ranunculaceous (such as *Helleborus fœtidus*) or Papaveraceous plant. Fresh.

Cladophora glomerata. Fresh.

Old internodes of *Tradescantia Virginica.* May to September. Fresh or in alcohol.

Stems, about ⅜ inch thick, of the Buckthorn, *Rhamnus Frangula.*

The best and most certain object upon which to follow easily the division of the nucleus and the cell,[1] is the staminal hairs of [the Spider-wort,] *Tradescantia Virginica*, already known to us, or of some nearly allied species. We must observe the hairs, however, in a stage of development, in which they are not yet fully formed, and are found in active cell-multiplication. For this purpose we take for investigation flower-buds, which, without stalk, measure between ⅛ and ¼ inch in height. We open these buds, and first pull off the anthers from the filaments with fine forceps. Then with the scalpel we make a cut across under the insertion of the ovary and the filaments, and lift this part entire out of the bud. We lay it in a drop of 3 p. c. solution of sugar, and then separate the filaments from their foundation with needles under the simple microscope. The ovary, and all the rest of the body of the flower, is removed from the preparation. We can observe the preparation directly upon the object-glass, as it remains alive under the cover-glass for a considerable time, and admits of the use of the strongest objectives. Or we place the preparation in a drop of the sugar solution on a cover-glass,

which we then lay upside down upon the edges of a moist chamber. We can thus retain the hairs in developmental condition for a half day or more, although the hairs, lying deeper in the suspended drop, will not admit of such high powers. In general we must take care, therefore, that the suspended drop is spread out flat.

The **resting nucleus** appears finely punctate (Fig. 113, *1*, bottom cell); but examined with stronger magnification, or in cells which have been brought somewhat under the influence of the surrounding fluid, we see that the minute granules are not isolated, but closely connected into rows, forming fine threads wound in and out: the entire nucleus thus forms a **network** or **framework** enclosed in a delicate **membrane**. Between the coils of the thread are distinguishable several **nucleoli** of various sizes. The nucleus is surrounded by a little cytoplasma, which is connected with the peripheral protoplasm by plasmic threads. This protoplasm contains, besides the scarcely distinguishable **microsomata** or **microsomes**, strongly refractive grains—**leucoplasts**. The nucleus getting ready for division increases in size, and from its fine framework forms gradually a coarsely granular thread. The nucleus then begins to elongate, and the coils of its thread arrange themselves in oblique direction, approximately parallel to one another (Fig. 113, *2*). At the same time the cell-plasma begins to collect at the two **poles** of the nucleus. We can easily observe all these progressive changes in one and the same cell, but this requires a comparatively long time and continuous, or only briefly interrupted, observation. The grains in the threads then become indistinct, this assumes gradually a homogeneous aspect, and lays its coils in definite fashions which are not in all phases easy to follow. In cells which are dying, the nuclear figures become for a short time clearer. Hence we can conclude from numerous observations that the coils, in the first place running obliquely, are folded in the equatorial plane of the nucleus, and at the same time are placed parallel to its long axis. The nuclear thread then segments at the places where it is folded, alike at the poles and the equator, and the nuclear figure then consists of individual pieces of thread which are bent in the equator like a hook. The next rearrangements are again uncertain. First sharply defined is the stage in which the thread-segments show themselves as separated into two bundles of straight segments, approximately of equal length, with their ends coming together

in the equator (*3*). If these daughter-segments are specially long they bend at their polar ends into a hook. The daughter-segments are equally numerous in the two opposite bundles. Since the stage in which we saw the coarsely granular, obliquely arranged threads (*2*) about an hour may have passed away. The segments appear almost homogeneous; but with stronger magnification we can recognise slight constrictions on the surface, which betray its construction out of successive discoid pieces. With

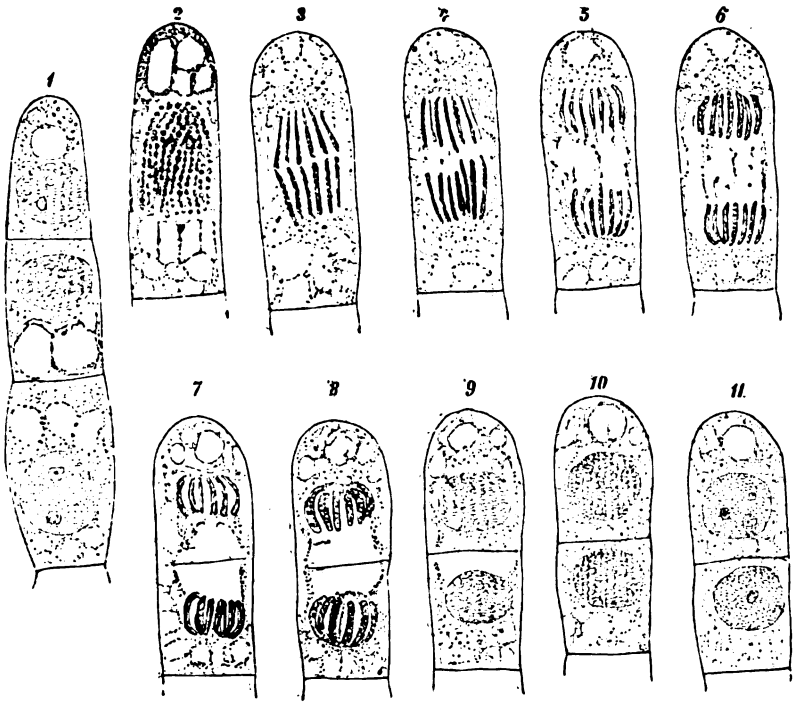


Fig. 113.– *Tradescantia Virginica*. Processes of division in the cells of the staminal hairs. Fig. *1*, with a resting nucleus in its lowest cell, and an upper cell which has just divided. Fig. *2*, with a coarsely granular nucleus showing oblique striation. Figs. *3–11*, successive stages of division followed in the same cell; *3*, about 10.10 o'clock; *4*, 10.20; *5*, 10.25; *6*, 10.30; *7*, 10.35; *8*, 10.40; *9*, 10.50; *10*, 11.10; *11*, 11.30 (× 540).

limited time we choose this last stage as the first for continuous observation. We have now to look for the separation of the two nuclear halves in the next few minutes, and this proceeds so quickly that we can see it take place. The two nuclear halves remove from one another in the longitudinal direction (*4*). Five

minutes later, the two nuclear halves are a noticeable distance from one another (*5*). The daughter-segments do not always separate from one another simultaneously, many remain behind and then hasten after the others. At the same time we see the daughter-segments, during their movement apart, bend at the poles, becoming according to their position somewhat shorter and correspondingly thicker (*5*). Between the nuclear halves remains a substance, transparent as glass, which is quickly increased in quantity by the immigration of the plasma-masses previously collected at the poles (*5* and *6*). In this transparent central mass a finer structure is not noticeable, but we can make out later that this mass is in fact differentiated into threads. It assumes gradually a barrel shape. From 25 to 30 minutes may have elapsed since the commencement of the separation, and we see appear in the equatorial plane of the central mass dark points arranged in rows. In the next moment these points fuse together, and we notice in their place a sharply defined dark line, the **young partition wall**. This has therefore proceeded from the small granules. These latter are microsomes, and form what we distinguish as the **cell-plate**. In the middle transparent protoplasmic substance, and at like distance from the two nuclear halves, a cell-plate is therefore first produced, and from this proceeds the young partition wall. If the central, barrel-shaped plasmic body has been formed so broad that it fills the entire cross-section of the cell, we see the partition wall at once joined on all sides to the wall of the mother-cell. If, on the other hand, the plasmic mass does not occupy the entire cross-section, it in all cases adjoins one side of the wall of the mother-cell, and we see it now, after the young partition wall has been formed on this side, move about inside the cell, so as gradually to come into contact in all directions with the wall of the mother-cell, and thus complete the parts of the edges of the partition wall which are still wanting. The central body withdraws therefore a little from the partition wall which is already present, and completes the parts which are wanting by forming cell-plate sections in their positions (*7–9*). During these processes we see the daughter-segments bend at their equatorial ends round towards the interior of the nucleus (*7*, *8*). The ends of the daughter-segments in this way come ultimately into lateral contact, and fuse together. Then once more only a single nuclear-thread is present, forming a coil. The nuclear-thread in the daughter-nuclei now again

begins to become finely granular, and, with stronger magnification, we note that it begins to change into a thin thread, bent zigzag to and fro (Fig. *9*, and the upper cell of *1*). The coils of this thread become longer, produce loops constantly increasing in number, these ultimately anastomose, and so form gradually (*10* and *11*) the stage which formed the starting point of our observations. At the same time the two **daughter-nuclei** increase in size, and we assume that they are nourished at the expense of the surrounding cytoplasm. They approach slowly nearer to the newly-formed partition wall. About an hour and a half after the commencement of the separation the formation of the daughter-nuclei is complete, and even nucleoli are visible in them (*11*). —Treatment with reagents gives, in the hairs of *Tradescantia*, results of little advantage. They are best fixed by 1 p. c. acetic acid, so that we can make use of acetic methyl-green in order to stain them at the same time. We can thus easily make out that the barrel-shaped mass of plasma, lying between the two nuclear halves, in which the partition wall is formed, and which appear clear as glass in the fresh state, really consists of threads, which join together the two rudimentary daughter-nuclei. We designate these threads as **connecting threads**; the innermost have a straight course, the rest describe curves so much the more marked as they approach the edge of the system. The granules which form the cell-plate, in case the proper stage is fixed, are very clearly visible, and appear with stronger magnification as equatorial swellings of the individual connecting threads.

In order quickly to obtain nuclei and cells in a fixed condition in the dividing state, we take for investigation the pollen mother-cells of the Monocotyledons. Especially to be recommended are many Liliaceæ; as *Fritellaria*, *Lilium*, *Alstrœmeria*, which possess especially large pollen mother-cells and nuclei. These genera stand so closely together in their relations that they can mutually replace one another. As we shall base our description upon *Fritellaria Persica*, we now state expressly that almost any species of *Lilium* and *Alstrœmeria*, and indeed most Liliaceæ and Amaryllidaceæ can replace it. It is at any rate of great advantage to select plants that unite in their inflorescences numerous flowers becoming successively ripe. Which buds conceal the desired developmental stage of the pollen mother-cells, we must find out by testing. We open a very young flower bud, take out an anther with the forceps, place it in a drop of acetic methyl-green, or

acetic gentiana-violet, lay a cover-glass upon it, and press upon it with a flat object, till the anther-cells are flattened and their contents evacuated. The evacuated contents are immediately fixed by the acetic acid and stained by the methyl-green or gentiana-violet, and we can see at once whether we have before us resting nuclei, or in a state of division. If the pollen mother-cells are already divided into four daughter-cells, or the young pollen-grains are already separated from one another, we must go back to younger flower buds. Whether we have to do with young pollen-grains, or with pollen mother-cells, we can easily recognise by the thick colourless walls of these latter. We go back to continually younger buds until we can see in the nuclei of the, as yet, thin-walled and connected mother-cells a fine thread-like coil, and a flat nucleolus lying against the wall of the nucleus. At this stage of the development the coiled thread contracts under the influence of the reagents, withdraws (Fig. 114, *a*) from the nuclear wall (this latter remaining uncoloured), and we can determine that this wall is a membrane formed by the surrounding cell-plasma (cytoplasm). The nucleolus we will here distinguish as a **lateral nucleolus (paranucleolus)**, because it occupies a peripheral position, and moreover otherwise comports itself somewhat differently from the ordinary nucleolus. This paranucleolus is characteristic of the nucleus of all pollen and spore mother-cells. The "coil" condition above observed has developed from that of the resting nucleus, which we should find in still younger flower buds, and which, just as we are accustomed to find it in the resting-nucleus, shows a fine framework and several nucleoli. With the coiled thread and paranucleolus we have reached the preparatory stage for cell-division, so we now pass step by step to older flowers. To fix them for the purpose of study we can either use acetic or formic methyl-green, or acetic or formic iodine-green, or acetic or formic gentiana-violet, or lastly picro-nigrosine. All these media fix immediately, and each has special advantages, so that we can with profit test them all. Preparations stained with gentiana-violet or with picro-nigrosine can be preserved in glycerine without decolorizing.—As a characteristic next succeeding state we meet with that (*b*), in which we see lying in the enlarged nuclear hollow, against the nuclear wall, segments of the nuclear thread, about 12 in number. These pieces of the thread appear distributed pretty uniformly on the nuclear wall. They are exclusively stained by treatment with acetic methyl-green, while the

nuclear hollow appears colourless. This latter, provided we have cut a comparatively young state, contains only homogeneous cell-sap; if we have an older stage the nuclear hollow is already traversed by a greater or less number of fine cytoplasmic threads.

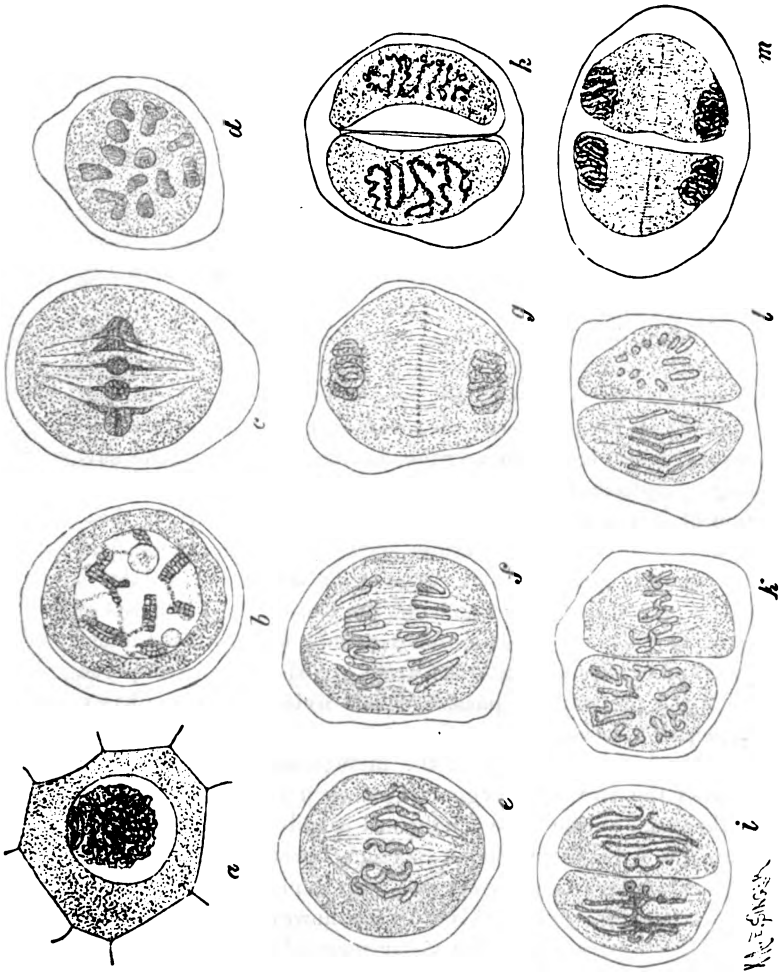


FIG. 114.—*Fritillaria Persica*, division of the pollen mother-cells. *a*, "coil" state; *b*, the segments in course of longitudinal division; *c*, the nuclear spindle in profile; *d*, seen from the pole; *e*, division of the nuclear plate; *f*, separation of the daughter segments; *g*, formation of daughter-coils and of the cell-plate; *h*, course of the nuclear threads in the daughter-nuclei; *i*, longitudinal elongation and formation of loops; *k*, nuclear spindle, to the right in profile, to the left seen from the pole; *l*, separation of the daughter segments, to the right in profile, to the left seen from the pole; *m*, granddaughter-coils, formation of the cell-plates (× 800).

The paranucleolus is feebly coloured, and attached somewhere to the nuclear wall, or to a segment. These segments have been formed from the nuclear thread, which we previously saw forming a coil. The thread had shortened considerably, thickening at the

same time, broadened into a band, and ultimately has separated into the said segments. In most favourable cases we can determine that each of these segments has split in the direction of its length into two equally thick daughter-segments (*b*). The daughter-segments separate in part from one another, and form Y-shaped or X-shaped figures. The next succeeding characteristic state presents us with the **nuclear spindle** (*c*). This shows equatorially placed, strongly stained segments, which form the **nuclear-plate,** and delicate unstained **spindle-fibres,** which converge towards the two poles of the nuclear spindle. To these spindle-fibres are attached the segments of the nuclear plate. The segments of the nuclear plate have the form of a Y lying down, and direct their two arms, following the spindle-fibres, towards the poles. Seen from the pole, the nuclear-plate presents an appearance as in Fig. 114, *d*.

The number of the segments regularly distributed in the nuclear-plate is in this plant usually 12. The segments of the nuclear-plate correspond with the longitudinally divided pairs of segments, which we previously observed attached to the nuclear-membrane. The nuclear membrane has been dissolved, the surrounding cytoplasm has penetrated into the nuclear hollow, and a portion of it has produced the spindle-fibres. Following these spindle-fibres the pairs of daughter-segments arrange themselves into the nuclear plate. Each segment of the nuclear-plate is therefore a pair of daughter-segments, the foot of the Y consists of the adjoining parts of the daughter-segments, usually fused under the influence of the reagent; the arms are the separated parts. With this the preparatory phases of cell-division, the **prophases,** are completed —Now begin the phases of separation and rearrangement of the daughter-segments, the **metaphases** of division. In this process the two sister-segments of each pair separate from one another, and at the same time wheel round polewards, so that they now lie with their convex portion towards the poles (*e*). These conditions are more rarely met with in preparations, as they are passed through very quickly; we can however see the further phases of the separation of the sister-segments, which appertain to the receding phases of division, the **anaphases.** Such a stage we see in Fig. 114, *f*. The daughter-segments follow the spindle-fibres, and, drawing closer together, reach their polar ends. Here their ends fuse together, and form a daughter-coil (*g*). All the conditions, from the beginning of the separation to the last-

observed stage, are often found together in the contents of one anther-loculus. While the daughter-segments move towards the poles, the spindle-fibres remain behind as connecting threads between them (*f*, *g*). The number of connecting threads is increased by the intercalation of new ones, and they ultimately form a barrel-shaped structure. Soon the connecting threads are only clearly marked in the equatorial parts, and in the equatorial plane itself appears, as thickenings of these threads, a row of granules, which represent the **cell-plate** (*g*). The cell-plate ultimately extends over the whole diameter of the cell, the elements of the cell-plate fuse, and form a partition wall, which halves the mother-cell into two daughter-cells. In each daughter-nucleus is formed a thin-threaded coil, the turns of which remain parallel to the original arrangement of the daughter-segments.

Later preparations show us that the nuclear thread in the nuclei of the daughter-cells again becomes coarser (*h*). Its coils, differing in this respect from the mother-nucleus, gradually elongate at right angles to their original direction, and form loops in the equator (*i*). The points of curvature at the poles and in the equator are broken through, the segments contract, and withdraw to the equator. Thus arises the **nuclear plate**, in which the spindle-fibres on both sides are very difficult to recognise (*k*, right-hand). The segments of the nuclear plate are arranged into a wreath (*k*, left-hand). The division of the two nuclei follows in the same, or in two planes cutting one another at right-angles, hence giving figures with two views, as in (*k*). The segments of the nuclear plate divide in the direction of their length, though this cannot be seen in preparations fixed in this way. Then, however, the daughter-segments withdraw from one another, and their reduced thickness bears witness to their having split (*l*). The subsequent processes correspond with those in the mother-cell. The two cells divide in the same way into four grand-daughter-cells, which either lie in the same plane (*m*), or cross one another at right-angles, according to the direction which the nuclear division took. The four grand-daughter-cells soon acquire their own walls, and are set free by solution of the wall of the mother-cell.

Preparations fixed in this way do not suffice for more careful researches upon nuclear and cell-division. For this purpose we prepare suitable material by laying the flower-buds of various ages in absolute alcohol. Preparations fixed with chromic acid, picric acid, or mixtures with chromic acid, are here, in general, inferior

to alcohol-material. Taking objects which must have lain at least three days in absolute alcohol, we rapidly prepare longitudinal sections through the anthers, and lay these in a solution of safranin in absolute alcohol,[2] first diluting the solution with about one-half distilled water. In a drop of this fluid upon an object-slide the sections can afterwards be examined, in order to determine approximately which stage of division they contain. In the safranin solution the sections have to lie for from twelve to twenty-four hours, and then they are transferred to absolute alcohol, and moved to and fro so long as they give off visible clouds of colour. We then place the sections in oil of cloves (better still in oil of marjoram), and as soon as they are completely saturated, in cold solution of gum-dammar (dammar dissolved in warm turpentine, and evaporated to a syrup), or in Canada-balsam (dissolved in chloroform or in turpentine), in which they remain unchanged. With accurate treatment the nuclear substance alone is stained; the spindle-fibres are only feebly marked in such preparations. The structural relations of the cytoplasma are most sharply defined in a filtered solution (as thick as syrup), of the clearest possible shellac in absolute alcohol. Canada-balsam clears the preparation even more than dammar solution. Gentiana-violet, with the same kind of treatment, gives nuclear colorations which are almost better than safranin.[3]—In order to make the spindle-fibres more visible, we lay a number of sections of the alcohol-material in very dilute hæmatoxylin (logwood) solution, made by dropping into a watch-glass full of water only a few drops of an old Grenacher's or Böhmer's solution of logwood. The sections must not however be placed directly out of the alcohol into the hæmatoxylin solution, but, in order that they shall have no precipitate formed upon them, must previously have passed through distilled water. In the logwood solution the sections remain for several hours, during which the degree of staining can be controlled by microscopical test; when the desired coloration is obtained, we put up the preparation in glycerine. In case of overstaining we remove the surplus of stain, before laying in glycerine, by means of water, in which the sections have to lie for a considerable time, or by means of an iron-alum solution. Overstained sections can also be treated with 70 p. c. alcohol which contains $\frac{1}{4}$ p. c. hydrochloric acid, and then washed in 70 p. c. alcohol, or water, containing a trace of ammonia; but this kind of treatment requires the greatest possible care. Far more beautiful logwood prepara-

tions, which in perfection are not inferior to safranin preparations, are obtained by transferring the sections stained in watery logwood into 70 per cent., and then into absolute, alcohol, thence into oil of cloves or lavender, and from this into gum-dammar solution or into Canada-balsam. The structural relations of the cytoplasma are most sharply defined in a filtered solution, as thick as syrup, of the clearest possible shellac in absolute alcohol. Into this solution the preparation, after staining, is transferred directly from the absolute alcohol, and is preserved in it for a long time unchanged. The sections need only to remain a short time in the alcohol and the volatile oil.—Instructive preparations are also quickly obtained by colouring the alcohol-material with fuchsine-iodine-green.[1] It is best to prepare a solution of fuchsine, and of iodine-green, each in 50 p. c. alcohol, pour the iodine-green into a saucer, and slowly add to it the fuchsine solution until the fluid has taken a distinct violet colour. The anther sections to be stained are placed on the object-slide in a drop of this fluid, which after the lapse of about a minute is run off by tilting the object-slide, and sucked up with blotting paper. A drop of glycerine is then placed upon the object, the sections arranged, and covered with a cover-glass. These sections show the cytoplasma red, the nuclear substance blue, the paranucleolus stained red; the preparations are exceedingly beautiful and instructive, though inferior in sharpness of delineation to safranin and good logwood-preparations. They can be closed with Canada-balsam, and subsequently with gold size. Canada-balsam however, as has been already mentioned (p. 230), is soluble in the oils used for homogeneous immersion; care should therefore be taken not to allow the oil to remain in contact with the balsam, and, after use, to wipe the oil off rapidly. As the Canada-balsam used in closing always runs under the cover-glass a little, when it is used the object need not be protected in any other way from the pressure of the cover-glass. If gold-size alone is used for closing, it is recommended first to draw two lines of gold-size across the object-slide with the camel-hair brush. These lines must be at such a distance that the cover-glass will rest with its two edges upon them. The cover-glass is first laid on when the lines are half dry. The line of gold size drawn round the edge of the cover-glass must be laid on several times, waiting till the previous layer is dry before putting on a new one, and using for the purpose very dilute gold-size, diluted with linseed oil The closure is complete when the preparation held up against the

light no longer shows lines of light at the edge of the cover-glass. The object can be protected from the pressure of the cover-glass in the simplest possible way by laying in the preparation hairs of sufficient thickness, or minute plates of mica. Or, for the protection of objects, before laying the cover-glass upon the object-slide, four spots of wax can be made upon it, by means of the wick of a small wax candle which is temporarily lighted and then put out again. Such wax candles can also be used in order to make a temporary closing layer of wax at the edge of a cover-glass already fixed by spots of wax at the corners.

In longitudinal sections through the anthers all the mother-cells are not found in the same stage of division. The stages succeed one another in the one or other direction, which is of considerable advantage to the observer.

In order to become acquainted with the processes as they take place in the pollen mother-cells of the Dicotyledons, we choose as best for examination one of the Ranunculaceæ or Papaveraceæ. In what follows we will refer to the stinking Hellebore, *Helleborus fœtidus;* in essentials all Dicotyledons offer the same conditions. In a flower-bud which, with stalk, measures from ⅓ to ½ of an inch in height we find, usually progressing from within outwards, all the stages of division represented in the successive anthers. Here, also, we crush the anthers in the fluids discussed in connection with *Fritillaria*, and obtain, moreover, the same figures as there, only smaller. After the first step in the division of the mother-nucleus, a cell-plate is produced in the connecting threads, but again dissolved, while the nuclei prepare for a second division. This second division, to distinguish from *Fritillaria*, corresponds here completely with the first. The pairs of nuclei are joined by connecting-threads. These four nuclei are arranged in the globular mother-cell at the four corners of a tetrahedron (Fig. 115, *A*), and connecting-threads arise free in the cytoplasma in all directions between the four nuclei. Hence to the two bundles of connecting-threads previously present four more are added.

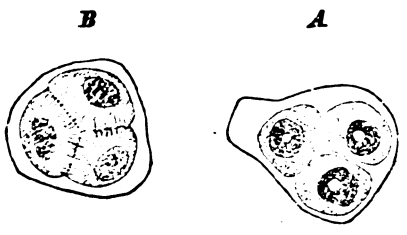


FIG. 115.—*Helleborus fœtidus.* Pollen mother-cells, at *A* in quadripartition; at *B*, after complete quadripartition (× 540). [Three only visible; the fourth is not in focus.]

In these six bundles cell-plates arise (*A*). These latter are clearly visible; the connecting threads, however, are to be seen only in the most favourable cases. The six cell-plates have the form of quadrants of a circle; they join one another in the interior of the mother-cell. Upon the thick wall of the mother-cell are produced six interior, somewhat projecting, ridges (*A*), and to these the cell-plates join with their outer edges. Cellulose walls are quickly formed from the cell-plates, and thus the mother-cell is divided into four tetrahedrally arranged daughter-cells (*A*). These four cells soon obtain their own walls, and become free, while the wall of the mother-cell is dissolved.

The plant upon which cell-division was first observed is *Cladophora glomerata*.[5] We have already made ourselves acquainted with its structure, and know that it is **multinuclear**. Its cell-division takes place without being accompanied by nuclear division. Each daughter-cell contains, therefore, naturally a number of nuclei, which can further multiply; therefore nuclear division and cell-division here appear completely independent of one another. We can here find cell-divisions at all hours of the day; often, however, search for them in vain. If we have found one, we can hope for others, for usually, if they are dividing at all, numerous cells of the culture tend to divide. We easily recognise the dividing condition, since the place of the partition wall in course of formation is marked by a brighter ring on the cell. The process[6] begins with a slight annular aggregation of cytoplasm at the mid-length of the cell. The chlorophyll-layer moves back proportionally. The foundation of the partition wall now shows as a sharp line. It projects as a ridge into the cell-cavity, and forces the chlorophyll-layer continually deeper. The only slightly marked annular aggregation of cytoplasm remains at its inner border. On both sides of the young partition wall, between the impressed chlorophyll-layer and the delicate ectoplasmic membrane, cell-sap collects; hence arises the colourless ring in a cell which is thus dividing. The chlorophyll-containing contents of the cell are ultimately cut through, and the diaphragm-like partition-wall completed in the middle so as to be closed. The segmented chlorophyll-containing contents of the cell remain for some time removed from the newly-formed cell-wall; but gradually approximate to it. The cross-wall thus formed is at first extremely thin, and is then thickened gradually by the two sister-cells.—The nuclei are too small to permit an insight into the peculiarities

of their processes of division. Their dividing states may be easily fixed with 1 per cent. chromic acid, but are, however, seldom met with.

All the processes of nuclear division combined with an internal thread-like differentiation are collected together under the term **indirect division**, as opposed to the **direct division**, which consists in a simple constriction of the nucleus. Such direct nuclear division is often found in the older cells of higher plants, and as an exceptional case in the actively growing internodal cells of the Characeæ [Stoneworts].[7]

For the observation of **direct nuclear division** in older cells the older internodes of *Tradescantia Virginica* are especially suited

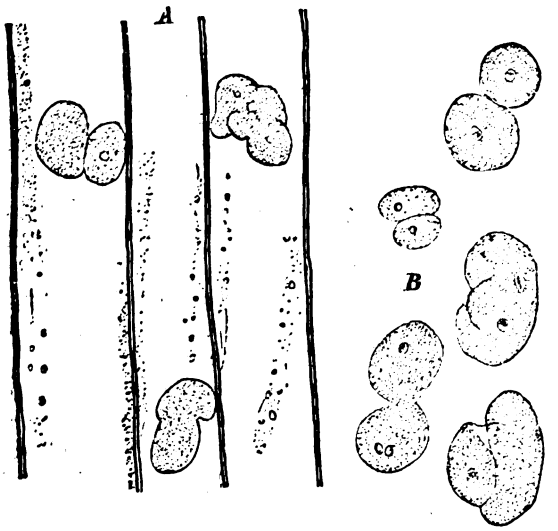


FIG. 116.—*Tradescantia Virginica*. Nuclei of older internodes in direct division. *A*, during life; *B*, after treatment with acetic methyl-green (× 540).

A longitudinal section, examined in water, shows them usually in considerable number (Fig. 116, *A*). The nuclei show their original contents, are, however, more or less irregularly constricted into several sections, of various size and form. If the constriction is unilateral, the nucleus appears kidney-shaped; with constriction all around, it shows as a figure of 8 or irregularly lobed. In many cases the segments have completely separated, and lie either by one another or at a greater or less distance. The number of the

thus segmented nuclei in one cell can amount to 8 or 10. They are of various sizes. The segments also can multiply by constriction. The nuclei in course of constriction are to be found in almost all the elements in the section, and best in the parenchyma of the pith. The thin-walled elements of the fibro-vasal bundle, which likewise contain the constricted nuclei, show besides very beautiful streaming of the protoplasm. The nuclei can be fixed very quickly with acetic methyl-green (Fig. 116, *B*). They then are very sharply defined.

In conclusion, we will call to our aid our strongest objectives, in order to approach a question the determination of which is of the greatest possible importance for the collective conception of the vegetable body. This relates to a reciprocal **inter-connection of the protoplasmic cell-bodies** of the plant, of such a kind that they form a single, continuous whole.[8] The most favourable objects for this study are provided by the **secondary cortex** of dicotyledonous plants, and amongt these we select specially the Buckthorn, *Rhamnus Frangula*. From a part of a stem, at least $\frac{1}{5}$ inch thick, we first remove the periderm with the razor, and then prepare delicate tangential longitudinal sections through the green cortex. We will use these sections in order to inform ourselves as to the structure of the secondary cortex, and for this purpose examine them in water. We direct our attention principally to the chlorophyll-containing bast-parenchyma, which we see to be composed of rectangular cells, chiefly elongated tangentially. These cells have more or less strongly thickened walls, penetrated by broader or narrower pits, in part so narrow that they are difficult to distinguish.[9] All these pits are without "borders." Besides the bast-parenchyma, above all will strike us the long bast-fibres, and the spindle-shaped cross-sections of the medullary rays. We now prepare new sections, likewise tangential longitudinal, through the secondary cortex, lay them upon a cover-glass, and place upon them a drop of concentrated sulphuric acid. After some seconds we immerse the cover-glass in a glass full of water, and wash the section rapidly, and as completely as possible. It can now be stained with watery aniline-blue, washed with water, and placed in dilute glycerine. Instead of watery aniline-blue, picric aniline-blue can be used with advantage. This is prepared by dissolving picric acid to saturation in 50 per cent. alcohol, and adding aniline-blue till the solution has a blue-green coloration. The investigation must be carried on with the strongest magnification;

and, where possible, with objectives for homogeneous immersion. The action of the acid is what is desired when the walls of the bast-parenchyma are so far swollen, that they show about the same diameter as the contracted cell-body. The middle lamellæ of the walls are likewise swollen, and it is this circumstance which makes the object so favourable for investigation. The contracted protoplasmic bodies are beautifully stained by the aniline-blue. The outlines of the individual plasmic bodies of the cells of the cortical parenchyma are smooth on those surfaces with which they bound cell-walls provided with very fine pits; they are provided with thicker or thinner processes where the adjoining cell-wall possessed broader pits. The processes of the plasmic body corres pond in the neighbouring cells. We first closely examine the swollen **closing membrane**, which separates two especially broad processes, directed towards one another. We find stretched between these two processes a number of extremely delicate, granular looking threads. These are plasmic threads, by which the neighbouring plasmic bodies communicate while in the living state. The outer threads of such a system are arched, and remind one, therefore, strikingly of the connecting threads which are extended between two sister-nuclei. Where the surfaces of two cells turned towards one another appear smooth, we usually find the middle layers of the cell-wall, through their entire extent, traversed by threads, which with very strong swelling of the wall are separated from the two plasmic bodies, or by slighter swelling are still connected with them. These threads are somewhat swollen in the middle, so that they appear spindle-shaped. In specially favourable cases the spindles appear interrupted in the centre, and their two halves joined together by extremely delicate, granular threads. But for such appearances it is often necessary to search long. In general, all plasmic bodies do not show their reciprocal union simultaneously; but only those which were not injured in any way in the preparation of the section, and which were quickly fixed by the sulphuric acid. The injured cells, or those which were not fixed quickly enough, have withdrawn their processes.—Those walls which appear to be pierced through their entire thickness by fine threads, produce the idea that we have in their interior exactly the same threads, within which in cell division the partition wall was deposited, which have therefore remained as connecting threads, in order to maintain the communication between the two cell-bodies.[10] In the formation of

broader pits, the communication later on exists only within these pits; however, that such direct union exists by means of protoplasmic processes between neighbouring cells appears now to be proved with certainty.

It is not difficult, also, to determine the connection by means of delicate plasmic threads in the endosperm of the Gramineæ. It is especially easy to see in tangential sections through the aleurone layer of *Triticum vulgare* and *Secale cereale*. In sections which, to follow another method of treatment, we have first soaked in alcoholic tincture of iodine, and afterwards in watery potassium iodide iodine, we can, after subsequent addition of sulphuric acid, bring the protoplasmic threads in the side walls into view in the most pregnant fashion.

NOTES TO CHAPTER XXXII.

[1] With this section compare Strasburger, *Zellb. u. Zelltheilung*, 3rd edit.; Flemming, *Zellsubst., Kern und Zelltheilung;* Strasburger, *Die Controversen der Kerntheilung.* The other literature is quoted in these works.

[2] Flemming, *Archiv f. mikr. Anat.*, Vol. XIX., p. 317.

[3] Flemming, *Zellsubstanz*, etc., p. 384.

[4] For the double staining of tissues, this stain was first proposed by J. Macfarlane. *Transact. of the Bot. Soc. Edinburgh*, Vol. XIV., p. 190.

[5] By von Mohl, in the year 1835. *Dissertation*, printed in *Flora*, 1837.

[6] Strasburger, *Zellbildung*, etc., 3rd edit., p. 203.

[7] Johow, *Botanische Zeitung*, 1881, col. 728. Strasburger, *Ueber den Theilungsvorg. d. Zellk.*, p. 98; also *Arch. f. mikrosk. Anat.*, Vol. XXI., where the literature is given.

[8] For general information, compare Strasburger, *Bau und Wachsthum der Zellhäute*, 1882, p. 246. For more special literature, Thuret et Bornet, *Etudes phycologiques*, p. 100. Frommann, *Stzber. der Jen. Gesellsch. f. Med. und Naturwiss.*, 1879, p. 55, and *Beobachtungen über Protopl. der Pflanzenzellen;* Tangl, *Jahrb. f. wiss. Bot.*, Vol. XII., p. 170; Russow, *Stzber. d. Dorpater naturf. Gesell.*, 1882, p. 350; Strasburger, *Stzber. d. Niederrh. Gesell. in Bonn*, Dec. 4th, 1882; Gardiner, *Quart. Journ. Microsc. Science*, 1882, p. 365; Hillhouse, *Bot. Centralbl.*, Bd. XIV., p. 89; Gardiner, *Quart. Journ. Micr. Science*, 1883, p. 301; and *Proceed. Royal Soc.*, 1883, p. 163; Schmitz, *Stzber. d. kgl. Akad. d. Wiss. in Berlin*, 1883, p. 219; Russow, *Stzber. d. Dorpat. naturf. Gesell.*, Sept., 1883; Gardiner, *Phil. Transactions of the Royal Soc.*, Part III., 1883, p. 817; Hick, *Journal of Botany*, 1884, pp. 33, 65.

[9] This object was recommended by Russow; the method of research is from Gardiner, *Phil. Trans.*, p. 821 *et seq.*

[10] Compare Strasburger, *Bau u. d. Wachsth.*, p. 248; and Russow, as cited above.

NOTES.

APPENDIX I.

ENGLISH AND METRIC WEIGHTS AND MEASURES

The following may be useful as an approximate transfer table.

LENGTH.

French.	*English.*
1 millimetre (mm.)	= $\frac{1}{25}$ inch.
1 centimetre (cm.) = 10 mm. . .	= $\frac{2}{5}$ inch.
1 decimetre (dm.) = 100 mm. . .	= 4 inches.
1 metre = 1000 mm. . .	= 39$\frac{1}{3}$ inches.

English.	*French.*
1 inch	= 25 mm.
1 foot	= 305 mm. or 30$\frac{1}{2}$ cm.
1 yard	= 0·91 metre.

WEIGHT.

French.	*English.*
1 gramme	= 15$\frac{1}{2}$ grains.
1 kilogram. = 1000 gram. . . .	= 32 oz. Troy.
„ „	= 35$\frac{1}{4}$ oz. Avoirdupois

English.	*French.*
1 oz. Troy	= 31 gram.
1 oz. Avoirdupois	= 28 gram.
1 lb. „	= 450 gram.

CAPACITY AND WEIGHT.

French.	*English.*
1 gram. = 1 cubic centimetre (cc.) . .	= 15$\frac{1}{2}$ grains.
1 litre = 1000 gram. or 1000 cc. or 1 kilog.	= 35$\frac{1}{4}$ oz. Avoird. or 32 oz. Troy.

English.	*French.*
1 pint = 20 oz. Avoirdupois . . .	= 567$\frac{1}{2}$ cc. or 567$\frac{1}{2}$ gram.

CAPACITY (VOLUME).

French.	*English.*
1 litre = 1000 cc. = 1 cubic decimetre .	= 1$\frac{3}{4}$ pint.

English.	*French.*
1 pint = 36 cubic inches . . .	= 567$\frac{1}{2}$ cc.
1 gallon = 8 pints	= 4$\frac{1}{2}$ litres.
1 cubic foot = 6 gallons	= 28$\frac{1}{3}$ litres.
1 cubic inch	= 16$\frac{1}{3}$ cc.

Microscopic measurements are usually reckoned in *micro-millimetres* (μ). 1 μ is $\frac{1}{1000}$ of a millimetre, and therefore is approximately $\frac{1}{25000}$ of an inch.

APPENDIX II.

LIST OF PLANTS USED FOR STUDY.

THE part of the plant required is carefully indicated in this list; likewise the state in which it should be taken, and the period at which it can be obtained. To these a few cultural notes are sometimes added.

Where the material is to be placed in alcohol, unless the word "absolute" is used, strong methylated spirit will serve, and is much cheaper.

For fixing cell-contents, the quantity of alcohol, etc. used should be at least 100 times the bulk of the object.

Stems, etc., which are much hardened in alcohol, can be rendered easy to cut by being placed for at least twenty-four hours in a mixture of half-and-half alcohol and glycerine.

Most of the living algæ here mentioned can be obtained from T. Bolton, Newhall Street, Birmingham.

A.

Acacia. Compound pollen-grains, 320. Flowers. Fresh, or in alcohol. Greenhouse shrubs.

Acer (Maple). Autumnal coloration of leaves, 43. Leaves in autumn. Fresh.

Aconitum Napellus (Monkshood). Structure of ovule, 327. Full-blown, or faded, flowers. Fresh, in summer. Hardy perennial.

(Other species of *Aconitum* will serve equally well.)

Acorus Calamus (Sweet Flag). Structure of root, 138. Roots. Fresh, or in alcohol. Hardy semi-aquatic.

Adonis flammeus. Colour-bodies of flowers, 42. Flowers. Fresh.

Æcidium Berberidis (Cluster-cup), 262. Infected leaves of Barberry, in May or July. Fresh, dry, or in alcohol.

Æsculus Hippocastanum (Horse-chestnut). Fall of leaves, 157. Base of leaf-stalk with piece of twig attached. Autumn. Fresh, or in alcohol.

„ Glandular hairs, 81. Winter buds. Preferably fresh, or in alcohol.

Agapanthus umbellatus. Development of anther and pollen, 316. Substitute for *Hemerocallis*, *q.v.*

Agaricus campestris (Mushroom). Structure, 200. Any time in the year. Fresh, or preferably in alcohol.

„ Fructification, 268. Any time in the year. Fresh.

Agave. Epidermis and stomata, 67. Leaves. Fresh. Any time. Greenhouse perennial. (Substitute for *Aloë*.)

Ailanthus glandulosa. Leaf-fall, 159. Leaf-bearing twigs. Fresh.

Alder, *see Alnus glutinosa*.

Alisma Plantago (Water Plantain). Structure of fruit, seed, and embryo, 343. Ripe and unripe fruits. Fresh. July and August. Native aquatic.

Allium Cepa (Onion). Structure of root, 136. Roots. Fresh, or in alcohol. Obtainable at any time by growing an onion in water in a hyacinth glass.

Alnus (Alder). Tannin reaction, 53. Twigs. Fresh. Any time.

Aloë nigricans. Epidermis and stomata, 67. Leaves. Fresh. Any time. Greenhouse perennial.

(Other species, or *Agave*, serve equally well.)

Alstrœmeria. Cell and nuclear division, 360. Flower-buds of various ages. Fresh; also in absolute alcohol. (*A. aurantiaca*, the commonest species, is a hardy perennial, flowering July to September.)

Althæa rosea (Hollyhock). Pollen-grains, 319. Flowers. Fresh. July to September. Hardy perennial.

Ampelopsis hederacea (Virginian Creeper). Autumn tints, 43. Leaves. Fresh. Autumn. Hardy climber.

Anabæna Azollæ. Structure, 216. Exists in the leaves of *Azolla carolineana*. Fresh. Any time. *Azolla* is a perennial greenhouse aquatic.

Anagallis (Pimpernel). Structure of ovary, 326. Flowers. Fresh, or in alcohol. Summer. *Anagallis arvensis* is a cornfield annual, with scarlet flowers; *Anagallis tenella* is a very pretty creeping native bog-plant.

Anaptychia ciliaris. Structure, 202. Thallus. Fresh; or dried, but soaked in water before use.

„ Fructification, 270. Fruiting thallus. Fresh; or dried, but soaked in water before use.

Aneimia fraxinifolia. Epidermis and stomata, 69. Leaves. Fresh. Any time. Various species of *Aneimia* are found in most fern-houses.

Antirrhinum majus (Snapdragon). Coloured cell-sap, 41. Flowers. Fresh. May to September. Hardy perennials.

Aristolochia Sipho. Structure of stem, 104. Young stems, $\frac{1}{8}$ to $\frac{1}{4}$ and $\frac{3}{8}$ inch thick. In alcohol; material to be put up in June. Hardy deciduous or half-evergreen, climber.

Arrowroot, East Indian and West Indian, for starch, 11. Shops.

Ash, *see Fraxinus excelsior*.

Aspidium Filix-mas (Male Fern). Fructification, 289. Fertile frond. Preferably fresh, but may be in alcohol. Late summer or autumn. Native fern.

Auricula, *see Primula*.

Avena sativa (Oat). Starch grains, 12. Grain. Fresh.

„ Structure of vascular bundle, 93. Stems. In alcohol. Spring or early summer. Can be grown in laboratory for the purpose.

„ Puccinia on, 264. Leaves or haulm. Fresh, dry, or alcohol. Summer.

Azalea. Compound pollen-grains, 320. Flowers. Fresh. (For substitutes *see Calluna*, *Erica*, *Rhododendron*.)

B.

Bacillus subtilis, 236. From infusion of hay. For method, *see* text.

Bacillus tuberculosis, 234. In the sputum (expectorations) of consumptives.

Bacteria, 221. To obtain materials, *see* text.

Barberry, *see Berberis vulgaris.*

Barley, *see Hordeum vulgare.*

Bean, *see Phaseolus vulgaris.*

Beech, *see Fagus sylvatica.*

Beet-root, *see Beta vulgaris.*

Beggiatoa alba, 232. In water containing decaying fresh-water algæ, or containing fragments of india-rubber tubing.

Berberis vulgaris, *see Æcidium Berberidis.*

Bertholletia excelsa (Brazil-nut). Albumen crystals, or crystalloids, 26. Nuts. Fresh.

Beta vulgaris (Beet-root). Structure of root, 45. Root. Fresh.

,, Sugar in root, 49. Root. Fresh.

Bracken-fern, *see Pteris aquilina.*

Brazil-nut, *see Bertholletia excelsa.*

Buckthorn, *see Rhamnus Frangula.*

Butomus umbellatus (Flowering Rush). Structure of ovary, 324. Fully developed flowers. Fresh, or in alcohol. Summer. A native perennial aquatic.

C.

Calluna vulgaris (Ling). Compound pollen-grains, 320. Flowers. Fresh, or in alcohol. July to September. A native sub-shrub.

Capsella Bursa-pastoris (Shepherd's Purse). Structure and development of embryo and seed, 338. Young to ripe fruits. Fresh. All summer. Common weed of cultivated ground.

Carrot, *see Daucus Carota.*

Celandine, *see Chelidonium majus.*

Ceratopteris thalictroides. Cultivation of spores and development of prothallus, 290 and 297*a*. Spores. Fresh, or preserved dry.

Cheiranthus Cheiri (Wallflower). Hairs, 72. Young leaves and buds. Fresh.

Chelidonium majus (Celandine). Structure of vascular bundles, 101. Stems. In alcohol. Spring and summer. Hardy herbaceous perennial.

Cherry. Substitute for Plum (*Prunus domestica*, *q.v.*).

Citrus vulgaris (Orange). Structure and development of fruit and polyembryony, 350. Ovaries and young fruits. Fresh. All the year round.

Cladophora glomerata. Structure, 203. Fresh material. Spring and summer.

,, Swarmspores, 248. For method of securing, *see* Text.

,, Cell-division, 368.

Clostridium butyricum, 223.

Club-moss, *see Lycopodium.*

Cluster-cup, *see Æcidium.*

Cowslip, *see Primula.*

Cucurbita Pepo (Cucumber, Melon, etc.). Movement of protoplasm in hairs, 35. Very young shoots. Fresh.

,, Pollen-grains, 320. Flowers. Fresh.

,, Structure of vascular bundles, 130. Stems about $\frac{1}{2}$ inch thick, cut about $\frac{1}{2}$ yard from apex. Fresh and in alcohol.

Curcuma leucorrhiza (East Indian Arrowroot). *See* Arrowroot.

Cytisus Laburnum (Laburnum). Structure of cork, 155. Fragments of bark from pretty old branches.

D.

Dahlia variabilis (Dahlia). Structure of tuber, 50. Tuber. Fresh. Any time.
,, Inuline, 50. Pieces of tuber placed in alcohol in or about October.

Date, *see Phœnix dactylifera.*

Daucus Carota (Carrot). Colour-bodies in root, 43. Root. Fresh.

Delphinium Ajacis (Larkspur). Structure of ovary, 322. Fading flowers. Fresh. Summer. A hardy annual. (As substitutes, *see Helleborus niger* and *H. fœtidus.*)

Delphinium consolida (Larkspur). Coloured cell-sap and colour-crystals, 42, 44. Flowers. Fresh. Summer. Hardy annual.

Dictamnus Fraxinella (Dittany). Development of oil-glands, 163. Leaf-buds and leaves. Fresh, or in alcohol. A hardy perennial. (A substitute for *Ruta graveolens, q.v.*)

Dittany, *see Dictamnus Fraxinella.*

Dracæna rubra (*Cordyline rubra*). Structure of stem, 96. Stems. Fresh, or in alcohol.

Drosera rotundifolia (Sundew). Digestive glands, 79. Leaves. Fresh. Summer. A native herbaceous perennial bog-plant. Can be grown in a greenhouse, in bog-moss, the pot standing in water.

E.

Echeveria. Wax-layer, 81. Leaves. Fresh. All the year round. Almost any species will do. All half-hardy evergreen perennials, largely used for borders in "bedding-out."

Elæagnus angustifolia (Oleaster, or Wild Olive). Hair-scales, 76. Leaves. Fresh. (Substitute for *Shepherdia canadensis, q.v.*)

Elder, *see Sambucus nigra.*

Epilobium (Willow-herb). Pollen-grains, 319. Flower. Fresh. (Substitute for *Œnothera biennis, q.v.*)

Epipactis palustris. Structure of ovary, 326. Faded flowers. Fresh. (Any orchid will serve about equally well.)

Equisetum arvense (Field horse-tail). Apical cell, 176. Young growing shoots. Fresh, or in alcohol. Spring.
,, Structure of stem, 180. Stems. Fresh, or alcohol-material.

Erica (Heath). Compound pollen-grains, 320. Flowers. Fresh. Any species will do, and hence obtainable nearly all the year round, either from open ground or greenhouse. (For substitutes, *see Calluna, Azalea, Rhododendron.*

Eucalyptus globulus (Australian Blue-gum). Wax-layer, 81. Leaves. Fresh. Half-hardy perennial evergreen tree.

Euonymus japonicus (Spindle-tree). Growing apex, 174. Terminal buds. Fresh, or in alcohol. All the year round. Ornamental evergreen shrub; half-hardy in the northern counties.

Euphorbia helioscopia (Sun-spurge). Starch-grains, 12. Stems. Fresh, or in alcohol. A native annual weed of cultivated ground.

Euphorbia splendens. Starch-grains and latex cells, 13. Stems. Fresh, or in alcohol. A hothouse evergreen shrub.

F.

Fagus sylvatica (Beech). Differing structure of leaves when shaded and exposed, 164. Leaves from centre and outside of tree. Fresh.

Ferns. Prothallia and sexual organs, *see Ceratopteris* and *Polypodium*.

,, Sporangia, *see Scolopendrium, Aspidium*.

,, Structure of growing apex of root, *see Pteris cretica*.

,, Structure of vascular bundles, *see Pteris aquilina*.

Fir, Scotch, *see Pinus sylvestris*.

Flag, Sweet, *see Acorus Calamus*.

Fraxinus excelsior (Ash). Leaf-fall, 159. Leafy Twigs. Fresh. Can be used as substitute for *Æsculus*.

Fritillaria imperialis (Crown Imperial). Structure. Pollen, 318. Young flowers. Alcohol-material.

Fritillaria persica. Cell and nuclear division, 360. Flower-buds of different ages. Fresh, and in alcohol. (As substitute, almost any species of *Fritillaria, Lilium, Alstrœmeria*, or other Liliaceæ or Amaryllideæ.)

Frog-bit, *see Hydrocharis*.

Fuchsia. Pollen-grains, 319. Flowers. Fresh. (Substitute for *Œnothera, q.v.*)

Funaria hygrometrica. Chlorophyll-bodies, 38. Leafy stems. Fresh. All the year round.

,, Sexual organs, 279. Male and female plants. Fresh, or in alcohol. Plants with the sexual organs and the sporogonia in all stages of development can be found nearly all the year round.

,, Structure of spore-capsule, 285. Fresh, or in alcohol.

Funkia ovata. Development of pollen, 316. Flower-buds of different ages. May. Fresh, or in alcohol. (Substitute for *Hemerocallis, q.v.*)

G.

Gall, Oak. Structure and tannin reaction, 51. Fresh, or dried.

Ginkgo biloba, see Salisburia adiantifolia.

Gleocapsa caldariorum, 218. Fresh. On walls, flower-pots, glass, &c., in greenhouses and fern-houses. All the year.

Gleocapsa polydermatica, 218. As substitute, *see G. caldariorum*.

Gloxinia hybrida. Embryo-sac, 334. Flowers. Fresh.

Gymnocladus canadensis. Leaf-fall, 159. Leafy twigs. A very ornamental hardy deciduous tree. Prefers shaded position. (Can be used as substitute for *Æsculus, q.v.*)

H.

Hart's-tongue fern, *see Scolopendrium*.

Hedera Helix (Ivy). Resin canals, 123. Young twigs. Fresh, or in alcohol.

Hellebore, *see Helleborus*.

Helleborus fœtidus (Stinking Hellebore). Cell and nuclear division and pollen-formation, 367. Flower-buds of various ages. Fresh, or in alcohol.

,, Structure of ovary. Flowers. February and March.

Helleborus niger (Christmas Rose). Structure of ovary. Flowers. January. (These two substitutes for ovary of *Delphinium, q.v.*)

Hemerocallis fulva. Development of anther and pollen, 311. Flower-buds of different ages. Fresh, and in alcohol. Summer. A hardy herbaceous perennial. (As substitutes, *Lilium*, *Funkia*, *Agapanthus umbellatus*, *Tulipa*, *Hyacinthus*.)

Hippuris vulgaris (Mare's-tail). Structure of growing apex, 170. Growing buds. Fresh, or in alcohol. Late spring or early summer. A native perennial herbaceous marsh or aquatic plant.

Hollyhock, *see Althæa rosea.*

Hordeum vulgare (Barley). Structure of growing apex of root, 183. Roots of plants grown in flower-pots.

Hyacinth, *see Hyacinthus.*

Hyacinthus. Structure of ovary, 324. Full-open flowers. Fresh. (As substitutes, *see Tulipa*, *Lilium*, or other Liliaceæ.)

„ Development of pollen, 316. (Substitute for *Hemerocallis*, *q.v.*)

Hydrocharis Morsus-ranæ (Frog-bit). Movement of protoplasm in root-hairs, 35. Young roots. Quite fresh. A native aquatic, with floating rosettes of leaves; easily grown in still water.

I.

Indian corn, *see Zea Mais.*

Iris florentina. Structure of leaf, 61. Leaves. Fresh and in alcohol.

„ Wax, 81. Leaves. Fresh.

„ Endodermis of root, 139. Roots. In alcohol.

„ Vascular bundle, 93. Leaves. In alcohol.

Iris germanica. Starch-builders (leucoplasts) and starch. Surface rhizomes. Fresh. Both species are hardy, herbaceous, more or less evergreen.

Ivy. *See Hedera Helix.*

J.

Juglans regia (Walnut). Leaf-fall, 159. Leafy twigs. Fresh. *See also* as in *Æsculus.*

L.

Laburnum, *see Cytisus Laburnum.*

Larkspur, *see Delphinium.*

Lathyrus (Sweet Pea, Everlasting Pea). Formation of pollen tube, 321. Freshly opened flowers.

Leptothrix buccalis, 232. In the "fur" on teeth.

Leucojum (Snowflake). Development of pollen-grains of, 318. Flower-buds of different ages. Fresh, and in alcohol. (As substitute for *Tradescantia*, *q.v.*)

Lilium candidum (White Lily). Stomata, 71. Fresh, or in alcohol.

Lilium (Lily). Development of anther and pollen, 315. Flower-buds of different ages. Fresh, and in absolute alcohol.

„ Structure of ovary, 324. Fully developed flowers. Fresh.

„ Cell and nuclear division, 360. Flower-buds of different ages. Fresh, and in absolute alcohol. (As substitutes, *Fritillaria*, *Alstræmeria.*)

Liverworts, *see Marchantia.*

Lupinus albus (Lupine). Aleurone-grains, 24. Seeds. Dry.

Lycopodium complanatum (Club-moss). Structure of stem, 149. Stems. Fresh, and in alcohol.

Lycopodium Selago (Club-moss). Structure of stem, 150. Stems. Fresh, and in alcohol.

Lysimachia (Loosestrife). Structure of ovary, 326. Fully developed flowers Fresh. (Substitute for *Primula*.)

M.

Maize, *see Zea Mais*.

Malva crispa. Pollen-grains, 319. Flowers. Fresh. (Substitute for *Althæa rosea*, Hollyhock, *q.v.*

Maple (*Acer*). Autumn coloration, 43. Leaves. Fresh.

Maranta arundinacea (West Indian Arrowroot). *See* Arrowroot.

Marchantia polymorpha. Vegetative structure, 194. Thallus. Preferably fresh, or in alcohol.

,, Reproductive organs and sporogones, 272. Receptacles. Fresh, and in alcohol. June to August.

Mare's-tail, *see Hippuris vulgaris*.

Matthiola annua (Ten-week Stock). Hairs, 73. Leaves. Fresh, and in alcohol. Late spring and summer.

Metzgeria furcata. Structure of thallus, 197.

Micrococcus Vaccinæ, 231. Found in vaccine lymph.

Mnium hornum. Reproductive organs and sporogonia, 277. May and June. Fresh, or in alcohol.

Mnium undulatum. Vegetative structure, 190. Fresh. (As substitutes, *Mnium hornum*, or *Polytrichum*).

Monkshood, *see Aconitum Napellus*.

Monotropa Hypopitys (Bird Rape). Structure of embryo-sac, 330. Flowers. Fresh. Found occasionally in woods, etc., especially under beech trees; flowers in July and August. It should be examined fresh, as it becomes brown and opaque in alcohol. It bears transport very well, and can be preserved fresh for some time in a glass of water.

Morchella esculenta (Morell). Vegetative structure and cell-contents, 269. Fresh or dry.

Morell, *see Morchella esculenta*.

Mosses, *see Mnium*, *Polytrichum*, *Funaria*.

Mucor Mucedo (Pin-mould). Structure and reproduction, 255. Found in a few days on a piece of damp bread placed under a bell-glass, or on fresh horse-dung similarly placed. For zygote production, see p. 256c.

Mullein, *see Verbascum*.

Mushroom, *see Agaricus campestris*.

N.

Navicula, *see Pinnularia*.

Nerium Oleander (Oleander). Structure of epidermis, 69. Leaves. Fresh. A greenhouse evergreen. Leaves and flowers more or less poisonous.

Nitella. Rotation of protoplasm in, 37. Fresh plants. *Nitella* can be grown in glass vessels of water, especially if fed with the culture-fluid given on page 208. Structure, 202*e*. Fresh.

Nostoc ciniflonum, 217. Fresh. Sometimes found in large olive-green masses on damp paths. In some parts of the country known as "witches' butter."

O.

Oak-gall, *see* Gall.

Oat, *see Avena sativa*.

Œnothera biennis (Evening Primrose). Pollen-grains, 318. Flowers. Fresh. Summer. (As substitutes, *Epilobium*, *Fuchsia*, *q.v.*) Structure of ovary, 337. Fresh.

Onion, *see Allium Cepa*.

Orange, *see Citrus vulgaris* (*C. Aurantium*).

Orchideæ, Ovary, 326, see *Epipactis*.

Embryo-sac, 333. Flowers some time faded. Fresh. (Substitute for *Monotropa*, *q.v.*)

Ornithogalum unbellatum (Star of Bethlehem). Structure of cell-walls of seed, 53. Seeds. Dry.

Oscillaria, 217. Stagnant water, muddy ground, etc.

P.

Pæonia (Pæony). Formation of pollen-tubes, 321. Flowers. Fresh. Pollen-grains grown in 5 p.c. sol. of sugar, and 1·5 p.c. gelatine.

Palmellaceæ, 219.

Pansy, *see Viola tricolor*.

Papaver Rhœas (Field Poppy). Structure of petals, 169. Petals. Fresh, or in alcohol.

Parmelia ciliaris, *see Anaptychia*.

Pea, *see Pisum sativum*.

Pear, *see Pyrus communis*.

Penicillium crustaceum (Blue Mould), 259. Obtained on a piece of moist bread under a bell-jar.

Peronosporeæ, *see Phytophthora*.

Phaseolus vulgaris (Bean). Starch, 10. Bean flour.

Phœnix dactylifera (Date). Structure of endosperm, 54. Date-stones.

Phycomycetes, *see Mucor Mucedo*.

Phytophthora infestans (Potato disease), 257. Diseased leaves of potato. Fresh.

Picea vulgaris. Female cones and fertilization, 307. Cones. Alcohol. Mid-June. Fertilization is completed in June; the exact date for the locality varies from year to year. Hence cones should be gathered daily from June 1, and the scales, separated from one another, placed in absolute alcohol. Before investigation the scales must be laid for at least 24 hours in a mixture of equal parts of glycerine and water.

Pinnularia viridis, 210. Fresh. Not infrequent in standing and running water

Pinus sylvestris. Bordered pits, 55. Pieces of old stem in alcohol.

,, Structure of stem and development of bordered pits, 114. Young stems, and pieces of outer part of old stems, cut in June or July, and laid in alcohol. To be placed in glycerine and alcohol before using.

,, Male flowers, 298. Male cones. Alcohol. May or June. Laid in glycerine and alcohol before using.

,, Female flower, ovule, 304. Young cones. Alcohol. May or June. Glycerine and alcohol before using.

,, Pollination. 306. Young cones as above, but fresh.

Pisum sativum (Pea). Structure of seed, and aleurone-grains, 16. Ripe peas. Dry.

Pleurosigma angulatum, 214.

Plum, *see Prunus domestica*.

Polypodium vulgare (Polypody fern). Structure of petiole, 148. Leaves. Fresh.

,, Prothallus and sexual organs, 291. *See* text.

,, Sporangia, 290. Fertile leaves. Fresh.

Polytrichum juniperinum. Structure of stem, 192. Stems. Fresh, or in alcohol.

,, Antheridia, 279. Plants in "flower." May. Fresh, or in alcohol.

Primrose, *see Primula*.

Primula. Ovary, 325. Flowers of any species.

Primula sinensis. Glandular hairs, 78. Leaf-stalks. Fresh.

Protococcus viridis, 214. On damp bark or walls. Fresh.

Prunus domestica (Plum). Structure of fruit, 347. Fruit. Fresh.

Pteris aquilina (Bracken-fern). Structure of vascular bundle, 145. Young leafstalks. Fresh, or in alcohol.

Pteris cretica. Structure of root-apex, 188. Roots. Fresh, or in alcohol. Very commonly cultivated in pots; and roots can be best obtained unbroken by turning the plant out of the pot containing it.

Puccinia graminis (Rust fungus), 262. Dry, or in alcohol. Found in summer on different kinds of cereals, and on *Triticum repens* (couch-grass).

Pyrola (Winter-green). Embryo-sac, 330. Flowers. Fresh. Herbaceous perennials, various species of which can be easily cultivated on a shady border, in sandy peat.

Pyrus communis. Stone-cells in fruit, 47. Fresh fruit.

Pyrus Malus (Apple). Structure of fruit, 348. Fresh fruit.

Q.

Quercus pedunculata (Oak), *see* Call.

Quercus suber (Cork Oak). Structure of cork, 156. Bottle cork.

R.

Ranunculus Ficaria (Pile wort). Structure of seed, 346.

Ranunculus repens (Creeping Buttercup). Structure of roots, 140. Roots. In alcohol.

,, Structure of vascular bundle, 100. Runners. In alcohol.

Rhamnus Frangula (Buckthorn). Inter-protoplasmic union, 370. Secondary cortex. Fresh.

Rhododendron. Pollen, 320. Flowers. Fresh. (For substitutes, *see Azalea, Erica, Calluna.*)

Ribes rubrum (Red Currant). Structure and development of cork, 156. Young and older stems. Fresh, or in alcohol. Best gathered in July.

Ricinus communis (Castor Oil), Aleurone grains, 25. Seeds. Dry.

Robinia Pseud-acacia. Leaf-fall, 159. Treat as in *Æsculus*.

Rosa semperflorens. Structure of prickles, 76, 77. Young stems. Fresh. Also leaves. Fresh.

Rose. Coloured cell-sap, 42. Petals. Fresh.

Rue, *see Ruta graveolens*.

Rumex patientia (a Dock). Glandular hairs, 79. Stems with sheathing stipules.

Rush, Flowering, *see Butomus umbellatus*.

Russula rubra, 266. Fresh, or in alcohol. (As substitute, *see Agaricus campestris.*)

Rust-fungus, *see Puccinia graminis*.

Ruta graveolens (Common Rue). Structure of leaf, 160 *et seq*. Leaves. Fresh. Obtainable all the year round. A hardy sub-evergreen.

S.

Saccharomyces Cerevisiæ (Yeast), 215. Can be grown in Pasteur's fluid.

Saccharum officinarum (Sugar-Cane). Stem. Frequently grown in hot-houses.

Salisburia adiantifolia. Autumn tints, 43. Leaves. Fresh. A hardy deciduous tree.

Salix Caprea (Goat Willow). Tannin, 52. Twigs. Fresh. Any time in the year. Twigs of other willows will do.

Sambucus nigra (Elder). Cork, 152. Twigs of various ages. Fresh, and in alcohol.

Scolopendrium vulgare (Hart's-tongue Fern). Structure of leaf-stalk and mid-rib, 148. Leaves. Alcohol.

„ Structure of leaf, and sporangia, 287. Fertile leaves. Alcohol.

Scorzonera hispanica (Salsify). Latex system, 103. Roots. Fresh, and in alcohol. A hardy kitchen-garden herbaceous plant.

Scotch Fir, *see Pinus sylvestris*.

Selaginella Mertensii. Structure and spore-production, 296. Fertile shoots. Dry, or in alcohol. This, or some similar species, is universally cultivated in plant-houses.

Shepherdia canadensis. Scale-hairs, 75. Leaves. Fresh, or in alcohol. Hardy deciduous shrub.

Shepherd's Purse, *see Capsella Bursa-pastoris*.

Siphoneæ, *see Vaucheria*.

Solanum tuberosum (Potato). Starch, 4. Tubers. Fresh. *See also Phytophthora*.

Sphagnum acutifolium (Bog-moss). Structure, 193. Plants. Fresh. Very commonly used in plant-houses.

Spindle-tree, *see Euonymus*.

Spirochæte plicatilis, 231. Water containing decaying algæ, especially *Spirogyra* and *Vaucheria*.

Spirogyra. Structure, 208. Living plants.

„ Conjugation, 246. Living plants. Summer. Plants in this state are recognisable by the crinkled yellowish look, and clinging together of the masses of threads. For the culture of *Spirogyra*, *see* p. 207.

Staphylea. Formation of pollen-tube, 321. Flowers. Fresh. Grown in 5 p.c. solution of sugar, and 1·5 p.c. gelatine.

Stinging Nettle, *see Urtica dioica.*

Stock, Ten-week, *see Matthiola annua.*

Sugar-Cane, *see Saccharum officinarum.*

Sweet Pea, *see Lathyrus.*

T.

Taxus baccata (Yew). Structure of root, 141. Roots. Fresh, or in alcohol.

„ Flowers and young fruit, 301. Flowers in March. Fresh, or alcohol-material. The female flowers should be collected towards the end of April, kept in absolute alcohol, and twenty-four hours before required placed in half-and-half alcohol and glycerine.

Thuja occidentalis. Growing apex of root, 185. Young roots. Best in alcohol.

Tilia parvifolia (Lime-tree). Structure of stem, 125. Branches and twigs. Fresh, and in alcohol. The latter best gathered in July.

Toadstool, *see Amanita.*

Torenia asiatica. Fertilization, 334. Flowers. To study fertilization, the flowers selected should be pollinized by hand a day and a half, or two days, before they are required. *Torenia* is a hot-house shrub, flowering in June or July.

Tradescantia virginica. Movements of protoplasm, 28, 34. Flowers. Fresh. *T. virginica* is a hardy herbaceous perennial, flowering from May or June to September.

„ Stomata, 65. Leaves. (*T. zebrina*, a common plant in plant-houses, can replace this.)

„ Structure of Pollen-grains, 316. Flowers and buds of different ages. Fresh.

„ Development of pollen-tube, 321. Freshly opened flowers. Grown in a solution of 5 p.c. sugar and 1·5 p.c. gelatine.

„ Cell and nuclear division, 356. Fresh flower-buds between $\frac{1}{8}$ and $\frac{1}{4}$ inch high. Stamens should be examined in 3 p.c. sugar solution.

„ Direct nuclear division, 369. Old stems. Fresh.

Tradescantia zebrina. Stomata, 66. Leaves. Fresh.

Trianea bogotensis. Rotation of Protoplasm, 37*a*. Fresh.

Triticum durum (Wheat). Starch, 11. Wheat grains. Dry.

Triticum vulgare (Wheat). Structure of grain: aleurone, 19. Wheat grains.

„ Structure of grain and of embryo, 346. Fresh grains.

„ Germination, 346*d*. Fresh grains.

Tropæolum majus (" Nasturtium," or Indian Cress). Colour bodies, 40. Flowers. Fresh.

„ Water-pores, 70. Fresh, and in alcohol.

Tulip, *see Tulipa.*

Tulipa. Development of pollen, 316. (Substitute for *Hemerocallis*, *q.v.*) Flowers. April and May. Fresh, and in alcohol.
,, Structure of ovary, 324. Old Flowers. Fresh, or in alcohol.

U.

Urtica dioica (Stinging Nettle). Stinging hairs, etc., 77. Young leafy stems. Fresh.

V.

Vallisneria spiralis. Movements of protoplasm, 36. Strong, rather old, leaves. Fresh. Very commonly and easily grown in aquaria.
Vaucheria sessilis. Structure and reproduction, 250. Strong plants taken from still or flowing water, and placed the day before wanted in a shallow vessel with fresh water.
Verbascum nigrum (Mullein). Coloured cell sap, 41, 74. Flowers. Fresh.
,, Hairs. Flowers. Fresh, or in alcohol.
,, Ends of vascular bundles. Flowers. Fresh, or in alcohol. A native herbaceous perennial, flowering in July and August.
Verbascum thapsiforme. Hairs, 75. Leaves. Fresh, or in alcohol.
Vinca major or *V. minor* (Periwinkle). Coloured cell-sap, 42. Flowers. Fresh.
,, Sclerenchyma fibres, 53. Stems. Fresh, or in alcohol. Native or introduced, perennial evergreen plants, flowering July to September.
Viola tricolor (Pansy). Hairs, 74. Flowers. Fresh, or in alcohol. Can be had from May to September.
,, Stipules of. Glandular hairs, 82*a*. Perfectly fresh.
Virginia Creeper, *see Ampelopsis hederacea*.

W.

Wallflower, *see Cheiranthus Cheiri*.
Willow, *see Salix*.

Y.

Yew, *see Taxus baccata*.
Yucca. Structure of ovary, 325. Ovaries. In alcohol.
Zea Mais (Maize). Structure of vascular bundles, 83. Young stems. In alcohol.

ADDITIONS.

Chara, e.g. *Charis fragilis*. Structure, 202*f*. Fresh.
,, Reproduction, 254*g*. Fresh.

Fucus vesiculosus (Bladder wrack). Structure, 202*a*. Fresh, or in alcohol.
,, Reproduction, 254*e*. Fresh, and in alcohol.
,, *platycarpus*. Reproduction, 254. Fresh, and in alcohol.

Hæmatococcus (*Protococcus*) *pluvialis*. Structure, 220. Fresh.

Pelargonium zonale. Leafstalks. Fresh; 82 note *a*.

APPENDIX III.

REAGENTS USED IN THIS WORK, AND HOW TO PREPARE AND USE THEM.

All the reagents in this list can be obtained ready made of Messrs. SOUTHALL BROS. & BARCLAY, Manufacturing Chemists, Birmingham, or of Dr. GEORGE GRÜBLER, Leipzig, Dufour-Strasse, No. 17. Or the materials can be obtained from the same source; and the student will find in this Appendix instructions how to prepare them for use. It is hoped that the instructions are sufficiently explicit; but the Editor will be glad to have errors or inefficient descriptions pointed out to him.

Percentage solutions are made either by weight or volume; or, if the metric system (see Appendix I.) is used, by either indiscriminately. Thus, 1 per cent. acetic acid in water is made by taking 1 volume acetic acid and adding to 99 volumes distilled water; 5 per cent. potash solution, by taking 5 gram. potash and adding to 95 cc. distilled water (1 gram. weight = 1 cubic centimètre volume).

A saturated solution can be secured by seeing that some of the salt, etc., is always lying undissolved at the bottom.

Further information as to the uses of the reagents will be found by reference to the General Index.

N.B.—For most purposes where alcohol up to 90 per cent. is used (*i.e.* "alcohol," not "absolute alcohol,") strong methylated alcohol will serve. Its alcoholic strength varies from about 85 to 90 per cent. Percentage compositions can therefore be made with it instead of absolute alcohol.

Approximately 50 per cent. alcohol = 56 parts strong meth. alc., 44 water.
60 per cent. alcohol = 67 parts meth. alc., 33 water.
70 per cent. alcohol = 78 parts meth. alc., 22 water.
82 per cent. alcohol = 91 parts meth. alc., 9 water.
(As this is a rather special strength, it is best prepared with absolute alcohol.)
90 per cent. alcohol = strong methylated alcohol.

It must, however, be remembered that methylated spirit is seldom or never free of acid, and for delicate work, therefore, the percentage composition should not be made with it.

Material fixed in absolute alcohol can be preserved for use in 70 per cent. alcohol.

A.

Acetic acid, Glacial.

Acetic acid, 1 per cent. For fixing the nucleus, especially in combination with methyl green or gentiana-violet.

Acetic acid, 2 per cent. For fixing and defining nuclei.

Acetic acid, 38 per cent. In Barfoed's sugar reaction. *See* p. 49.

Acetic acid is an excellent clearing reagent, for rendering tissues transparent, and showing up the cell-walls. It is likewise used to distinguish crystals of oxalate of lime, as they are not soluble in it.

Acetic acid and Gentiana-violet. *See* Gentiana-violet.

Acetic acid and Methyl-green. *See* Methyl-green.

Agar-Agar. Obtained from *Eucheuma gelatinæ*, or *Gigartina speciosa*, is used in the East in the place of ordinary gelatine, for preparing soups and jellies. It bears, without liquefying, higher temperatures than ordinary gelatine. *See* also footnote to page 241.

Alcanna root and tincture. Used for resin reactions. The alcoholic tincture of alcanna is added to so much water that the resin will not dissolve in it, or a thin chip of alcanna (Alkanet) root, washed in water to remove dust, can be placed with the preparation, and dilute alcohol run under the cover-glass. The resin drops colour deep red in two or three minutes. The coloured drops are dissolved in strong alcohol. Alcanna-tincture also stains protoplasm pale rose-red, from a quarter to half an hour being needed. It is often used to identify the ground substance of oil-containing seeds.

Alcohol, Absolute. Where the alcohol should contain a very specific proportion of water, it is better to use absolute alcohol diluted, as ordinary spirit varies slightly in strength, and almost always contains acids. Thus, material preserved for working with carbonate of lime crystals (cystoliths) always shows these dissolved if kept in ordinary spirits. Where expense is an object, methylated spirits,—only about one-tenth the price,—can however for most purposes be used. (See under the special heads in Appendix II.)

Alcohol, 50 per cent. Used with alcanna, for resin reaction. *See* 119; also for making picric alcohol, and picric aniline-blue. *See* 370.

Alcohol, 60 per cent. *See* 234.

Alcohol, 70 per cent. Used in treating overstained preparations. *See* 365.

Alcohol, 82 per cent. Used to harden celloidin in. *See* 329.

Alcohol, Acidulated. 70 per cent. alcohol + 0·5 per cent. hydrochloric acid. Used in overstaining. *See* 206.

Alcohol and Glycerine, half and half.

Alcohol, Methylated.

Alcohol, Picric. Picric acid dissolved in 50 per cent. alcohol. An exceedingly good fixing and staining reagent for filamentous algæ.

Alum, watery solution.

Alum-carmine, Grenacher's. *See* Carmine.

Ammonia, strong watery solution. Used often instead of potash, for clearing tissues, etc. Also, after the use of nitric acid, to produce the yellow colour in protoplasm, known as the Xanthoproteid reaction. By the same reaction the middle lamella of thick-walled tissue is stained yellow. Ammonia is also of great service in softening dry herbarium material, preparatory to microscopical examination.

Aniline-blue, watery solution. Used for staining the callus of sieve-plates in sieve tubes.

Aniline-blue (*Hoffmann's Blue*), dissolved in 50 per cent. alcohol and containing 1 per cent. acetic acid, can be used for the same purpose. It likewise stains protoplasm and not the cell-wall, as the colour can be removed from the latter by washing in water and mounting in glycerine. Alcohol material must be washed with water before staining. *See* also Methyl-blue (or Methylene-blue).

Aniline-blue with picric acid. *See* Picric aniline-blue.

Aniline-green (*Acetic*). Dissolve aniline-green in 1–2 per cent. solution of glacial acetic acid in distilled water until the solution is of a clear blue-green colour. Especially good for fixing and staining the nucleus in its stages of division. The colour is not permanent.

See also Methyl-green.

Aniline oil. See Phenylamine.

Aniline sulphate or *chloride.* Test for lignin. In dilute watery, or in alcoholic solution, alcoholic chloride being best. Treat the section first with this, and lignified walls are stained yellow. If the section is subsequently treated with dilute sulphuric or dilute hydrochloric acid, the colour is deepened. Or a mixture of the solution with $\frac{1}{10}$ its bulk of sulphuric or hydrochloric acid can be kept.

B.

Beale's carmine. See Carmine.

Bismarck brown. Dissolved in hot water or dilute alcohol. Used for staining Bacteria, 230.

Borax-carmine (*Grenacher's*). 2–3 per cent. carmine dissolved in solution of 4 per cent. borax in water; dilute with an equal volume of 70 per cent. alcohol, and filter after allowing it to stand for some time.

Borax-carmine (*Thiersch's*). 4 parts borax dissolved in 56 parts distilled water; to this solution 1 part carmine is added; afterwards, one volume of this is mixed with two volumes absolute alcohol, and filtered (*Arch. für mikr. Anat.* i., page 149). This fluid stains somewhat slowly, but very beautifully. Preparations are best overstained, and then laid for some time in a watch-glass in 50 or 70 per cent. alcohol containing a drop of hydrochloric acid (*see* Alcohol, acidulated). Used especially for nuclear staining. Preparations must be preserved in glycerine, or glycerine jelly.

C.

Camphor. For use, *see* p. 308.

Canada balsam. Dissolved to the thickness of syrup, in turpentine, chloroform, benzole, or xylol. It can be obtained dissolved in turpentine in metal tubes like those used for liquid colours, and is very convenient in this form.

Carbolic acid. In alcoholic and watery solutions, used for clearing preparations, and is often better than potash.

Carbolic acid in hydrochloric acid. Dissolve carbolic acid in warm hydrochloric acid, and, while the mixture is cooling, add sufficient hydrochloric acid to dissolve any precipitate that may be formed. Lignified tissues treated with this reagent and exposed to sunlight, show a greenish-yellow or blue-green colour.

Carbon bisulphide. Solvent for sulphur grains, in *Beggiatoa alba.*

Carmine. Solutions of carmine usually colour diffusely; but the nuclei usually show very well stained if the preparations are afterwards treated for some time with acidulated alcohol (*q.v.*), or with acidulated glycerine, *i.e.* glycerine containing ½ per cent. hydrochloric acid.

Carmine, Alum (*Grenacher's*). A 1–5 per cent. watery solution of common or ammonia-alum is boiled for from ten to twenty minutes with ½–1 per cent. powdered carmine, and, after cooling, is filtered. A trace of carbolic acid is added. (*Archiv. f. mikr. Anat.* xvi., p. 465.)

Carmine, Ammonio-acetic (*Hoyer's*). 1 gram. carmine is heated in a sandbath in about 1–2 cc. strong ammonia solution and 6–8 cc. distilled water till the excess of ammonia is volatilized. When it forms only small bubbles the ammoniacal combination commences to decompose, and the solution becomes a clear red colour. After cooling, the precipitate is filtered off, leaving the fluid perfectly neutral. To this liquid add four to six times its volume of absolute alcohol; a clear red precipitate is formed, which can be filtered out and preserved. When required, this powder can be dissolved in water, and the solution is made permanent by the addition of 1–2 per cent. chloral hydrate. (*Biol. Centralb.* ii., p. 18.)

Carmine, Ammonio-acetic (*Hamann's*). To an ammoniacal solution of carmine, acetic acid is added until a precipitate begins to be formed. Filter the fluid before using. By the addition of 1 to 2 per cent. chloral hydrate it is made more permanent.

Carmine, Beale's. 0·6 gram. powdered carmine is dissolved in 2·3 cc. concentrated ammonia. At the end of an hour a mixture of 66 cc. water, 47·5 cc. heavy glycerine, and 19 cc. absolute alcohol is added to it. Mix, and after some time filter. (*How to Work with the Microscope*, 4th edit. p. 109.)

Carmine, Borax. *See* Borax-carmine.

Carmine, Picric. Dissolve Hoyer's carmine (*see* above) in a saturated watery solution of picrate of ammonia.

Cedar, Oil of. Used for treating preparations of bacteria, prior to mounting in Canada balsam or dammar. *See* p. 230.

Celloidin. A form of collodion. Use of, 329.

Cherry-wood extract. Twigs of cherry, rejecting the thin, green parts, are cut up into thin shavings, steeped in absolute alcohol for twenty-four hours, to remove the chlorophyll as much as possible. The shavings are now shaken free of the discoloured alcohol and steeped in a new supply, and allowed to remain for several days, being frequently stirred. This fluid is then filtered, and evaporated down until a fragment of very coarse unbleached blotting-paper moistened with it, and subsequently with hydrochloric acid, quickly becomes a deep violet colour. The residual fluid thus obtained is brown, and smells like camphor. Preserve in a well-closed bottle. Reagent for lignin, 59.

Chloral-hydrate. Used as a clearing reagent, especially for pollen-grains, 319, 320.

Chloroform. Solvent of fat and etherial oils.

Chlorzinc iodine (Iodized chloride of zinc, Schultze's solution). Zinc is dissolved in pure hydrochloric acid, and the solution evaporated (metallic zinc being kept in it during the process) to the consistence of strong sulphuric acid; in this is dissolved as much iodide of potassium as it will take up, and finally as much metallic iodine as it will dissolve. (Nägeli: *Sitzber. d. Kgl. Akad. d. Wiss.*, 1863, p. 383). Chlorzinc iodine is the simplest reagent for cellulose.

Chlorzinc iodine and iodine. Dissolve iodine in the chlorzine iodine till a precipitate begins to be formed. This fluid stains the callus of sieve-plates a deep brown colour.

Chrom-acetic acid, 1 per cent. Chromic acid 0·7 per cent., acetic acid 0·3 per cent., in water. Used for "fixing" algæ. Time taken, up to 24 hours.

Chromic acid, 0·5 per cent. For fixing bacteria.

Chromic acid, 1·0 per cent. For fixing *Nitella*, filamentous algæ, etc. 12 to 24 hours.

Chromic acid, 20 per cent. Used in preparing skeletons of diatoms, 213.

Chromic acid, 25 per cent. Dissolves membrane of pollen-grains, 318.

Chromic acid, Concentrated. Dissolves the middle lamella of lignified tissue, 59. Does not dissolve cork, 155. Skeletons of diatoms, 213.

(*All these Chromic Acid solutions are in Water.*)

Cloves, Oil of. Used for clearing sections prior to mounting in Canada balsam, etc.

Copper, Acetate. Used in Barfoed's sugar reaction, 49.

Copper, Ammon-oxide (cuproxide ammonia, ammoniacal cupric oxide). Oxyhydrate of copper is carefully precipitated from the sulphate by a dilute solution of ammonia; the clear green precipitate, separated and washed, is added while still moist to strong ammonia, in which, upon slightly warming, it is dissolved. Upon cooling, crystals of sub-sulphate of copper and ammonia fall to the bottom. The filtered liquid contains only the ammoniacal cupric oxide in solution. It must be kept in bottles of dark glass, or in the dark (Schweitzer, *Vierteljahrsschr. d. naturf. Gesell. in Zurich*, Bd. II. 1856). It can also be prepared by digesting copper turnings in an open bottle with the liquor ammon. of the Pharmacopœia. As it is very easily decomposed by light, it is perhaps best prepared fresh, when required. It is fit for use only so long as it rapidly dissolves cotton-wool.

Copper, Sulphate. Used in Fehling's solution for sugar, 48.

Corallin solution. Dissolved in water with the help of 30 per cent. its own weight of carbonate of soda. To prevent its altering, a little camphor can be added to the solution.

Constantly used for staining and differentiating mixed tissues. Specially stains lignin, sieve-callus, and starch.

Crystal Palace glass cement, or other similar cement, such as Coaguline. For fixing card labels on object-slides.

D.

Dammar, Gum. Dissolved in warm turpentine, and evaporated to the thickness of syrup. Can be obtained in tubes ready for use.

Diamond-fuchsin-iodine-green. Make a solution of fuchsin and of iodine-green in 50 per cent. alcohol; pour the iodine-green into a saucer and slowly add to it the fuchsin solution till the fluid has taken a distinct violet colour. Used for nuclear staining. *See* p. 366. Preparations can be mounted in glycerine.

Diphenylamine. 0·05 gram. in 10 cc. pure sulphuric acid. Used as reagent for nitrates and nitrites. *See* p. 49.

E.

Eau de Javelle (Potassium hypochlorite).

Eau de Labarraque (Sodium hypochlorite).

I give potassium hypochlorite the preference, though the two differ little in their action. It is best to prepare the Eau de Javelle yourself, by mixing 20 parts of the officinal (25 per cent.) chloride of lime with 100 parts water, allowing it to stand some time, and adding a solution of 15 parts pure potash in 100 parts water. After allowing it to stand for one or several hours the mixture is filtered, and the filtrate used. Should lime still remain in the solution, and as a result the drops brought into use form in the air a skin of crystalline carbonate of lime, this is easy to remove by adding a few drops of potash solution and filtering off the precipitate.

Egg, White of. Used, diluted with water, and with the addition of a little camphor, for observations in the embryology of Gymnosperms. *See* p. 308.

Enclosing (or mounting) fluid. Hoyer's, for Aniline preparations. A tall glass vessel with a wide neck is filled up to two-thirds with gum-arabic, in selected clear pieces. The vessel is then filled up to the neck with a solution of 50 per cent. acetate of potash, or with a watery solution of acetate of ammonia containing, to each 20 gram., 10 gram. of caustic ammonia neutralized by a sufficient quantity of acetic acid. The gum is dissolved in a few days, if the vessel is often shaken, and forms a syrupy fluid, which is filtered through thick swansdown—a process taking about 24 hours. *Biol. Centrbl.*, Bd. II. p. 23.

Enclosing fluid for Carmine and Hæmatoxylin (Logwood) preparations. This is prepared as above, excepting that, instead of acetate of potash or of ammonia, a concentrated solution of chloral hydrate, to which is added 5–10 per cent. glycerine, is used. After some time this fluid may become turbid, and it is then necessary again to filter it.

Preparations mounted in either of these fluids require no further enclosing.

Eosin. Strong solution in alcohol. Stains protoplasm deeply. Especially useful for sieve-tubes.

Eosin, Watery. Acts in much the same way.

Ether. As a reagent, used as a solvent for fat and ceric acid. Used also to dissolve cake celloidin. *See* p. 329.

F.

Fehling's Solution. For preparation, *see* p. 48. A reagent for glucose.

Formic gentiana-violet. *See* Gentiana-violet.

Formic methyl-green. *See* Methyl-green.

Fuchsin (Magenta). In 100 gram. of a 5 per cent. watery solution of carbolic acid is dissolved 1 gram. fuchsin (magenta), and then 10 gram. alcohol added. Filter. This fluid keeps well. It is advisable to warm the fluid in using it.

Used for staining tubercle-bacilli, 235.

Fuchsin. Dissolved in half-and-half alcohol and water. Sections treated previously with alcohol and stained with this, show up the structure of thickened cell-walls. Stained, and washed in absolute alcohol, the colour is removed from all excepting corky walls; very briefly washed, it is left also in lignified membranes.

Fuchsin with iodine-green. *See* Diamond-fuchsin iodine-green.

G.

Gelatine. Used in cultivation of pollen-grains, 321.

Gentiana-violet. Very dilute watery solution stains decolorized chlorophyll-bodies, and other chromatophores. Used also for staining bacteria, 222, 229.

Gentiana-violet, Acetic. Gentiana-violet is dissolved in 1 per cent. solution of acetic acid till the solution has a deep violet colour.

Gentiana-violet, Formic. Prepared with 1 per cent. solution of formic acid, as above.

These two stains are invaluble in preparations to show nuclear figures. They keep in glycerine.

Gentiana-violet in aniline water. For preparation, *see* pp. 234–5. Used for staining bacteria in the tissues.

Glycerine. Used also for clearing tissues by heating, 232, 342, and for dehydrating, 289.

Glycerine acidulated. Used like acidulated alcohol, *q.v.*

Glycerine, concentrated. An invaluable mounting medium.

Glycerine, diluted. Two parts glycerine, one part water.

Glycerine and alcohol. Half and half. Used especially for softening hard alcohol material. Preparations showing fixed cell-contents are best not transferred direct from alcohol to glycerine, but placed in a mixture of alcohol and glycerine allowed slowly to concentrate.

Glycerine-gum. 10 gram. gum-arabic, 10 cc. water, 40 to 50 drops glycerine. (Dippel, II. Aufl., Bd. I., p. 773.) Used for embedding in section-cutting, 192, 338.

Glycerine-jelly (Kaiser's). One part by weight of finest French gelatine is softened for about two hours in six parts by weight of distilled water. To this is added 7 parts by weight of chemically pure glycerine, and to each 100 gram. of the mixture is added 1 gram. concentrated carbolic acid. It is then warmed for from 10 to 15 minutes, while continually stirring, till all the flocculence which the addition of the carbolic acid caused has disappeared. While still warm, it is filtered through the finest cloth of spun-glass, which has been previously washed in distilled water, and laid in the funnel while still damp, (*Bot. Centralb.*, Bd. I., p. 25). Can be obtained of E. Kaiser, Berlin.

Gold-size. Finest English.

Gum-arabic. Use in making Enclosing fluids etc., and for embedding.

Gum-arabic. 10 per cent. clear filtered solution. Used for slackening movements of spermatozoids, 293.

H.

Hæmatin-ammonia. For preparation and method, *see* pp. 205–6. Used for staining cell-contents of filamentous algæ, which have been fixed in picric acid only. Troublesome, but often exceedingly beautiful.

Hæmatoxylin (Logwood). Böhmer's. Dissolve 0·35 gram. hæmatoxylin in 10 gram. absolute alcohol, and add this solution drop by drop to a second solution of 0·1 gram. alum in 30 gram. distilled water until a beautiful blue-violet colour is produced.

Hæmatoxylin (Logwood). Delafield's or Grenacher's. Prepare,—

(1) Saturated solution of hæmatoxylin crystals in absolute alcohol.

(2) Saturated solution of ammoniacal alum crystals in distilled water

Take 4 cc. of (1) and mix with 150 cc. of (2). Allow it to stand in the light for a week, filter, and add to it 22 cc. glycerine and 25 cc. methylated alcohol. Before use it is best to allow it to stand for some time till any precipitate has time to settle.

These solutions stain best if old, and should be used very dilute, *i.e.*, a few drops in a watch-glass of water. Alcohol preparations must previously be placed in water. All acids must be avoided, though ¼ per cent. hydrochloric acid is useful in case of overstaining (*see* pp. 205 and 365).

Logwood is invaluable for staining cell-contents, nuclear figures, etc.

Hydrochloric acid, ¼ per cent. in 70 per cent. alcohol. Used in restoring overstained logwood preparations. *See* p. 365.

Hydrochloric acid, ½ per cent. in 70 per cent. alcohol. Used in restoring overstained carmine preparations. *See* p. 206.

Hydrochloric acid, 10 per cent. in water.

Hydrochloric acid, 30 per cent.

Hydrochloric acid, Concentrated.

I.

Iodide of zinc in glycerine. A concentrated solution of pure dry iodide of zinc in pure glycerine. After filtering, if necessary, evaporate in a water-bath to the refractive index 1·518, for the D line of the spectrum. Used as an immersion fluid.

Does not attack balsam used as setting of the objective lenses.

Iodine, in alcohol. Officinal tincture of iodine, diluted with alcohol to a dark sherry colour. Or iodine dissolved in alcohol to the same tint.

Iodine, in chloral. Solution of 5 parts chloral hydrate in 2 parts water, with a little iodine solution added. Used for decolorizing chlorophyll-bodies, and showing the contained starch-grains.

Iodine, in glycerine. Iodine dissolved in glycerine, and water added to dilute it. When undiluted, can be used to show minute grains of starch, *e.g.* in growing points, by carefully heating the preparation in a drop.

Iodine, in potassium iodide. Take 5 cg. iodine, 20 cg. potassium iodide, and 15 cc. distilled water.

Iodine, and sulphuric acid (colours cellulose blue). Best obtained with potassium-iodide iodine, and sulphuric acid diluted with half its volume of water, *i.e.* 2 vols. acid to 1 vol. distilled water.

Iodine in water.

Solutions of iodine should be kept in darkness, or else in coloured glass bottles. Or the stock can be kept in a dark cupboard, and that in use replaced every month or so.

Iodine. See also Chlorzinc iodine.

Iodine-green. Used especially in double-staining. *See* 92, 93.

Iodine-green, Formic. In 1 or 2 per cent. formic acid iodine-green is dissolved until the fluid appears a deep blue-green colour.

Iodine-green, Acetic. In 1 or 2 per cent. acetic acid, etc., as above.

Iron-alum solution. Used to remedy overstaining with logwood, 365.

Iron-perchloride solution.
Iron-sulphate solution. } Used in tannin reactions, 52. A dilute watery solution, to which a drop of nitric acid may be added.

L.

Lavender, Oil of. Used as a clearing reagent, instead of oil of cloves, before mounting in dammar or Canada balsam, 366.

Lemon, Oil of. Used as a clearing reagent for pollen-grains, 319, 320.

Lime, phosphate. Used in culture fluid for fresh-water algæ, 208.

Lime, sulphate. The same.

Linseed oil. Used for diluting gold-size, 366.

Logwood. See Hæmatoxylin.

M.

Macerating mixture, Schultze's. Several methods for using this important reagent have been suggested.

(1) Place in a wide test-tube some pieces of chlorate of potash, and pour over them sufficient strong nitric acid to completely cover them; then lay in the fluid longitudinal sections of the material and warm over a flame till gas is actively evolved. Allow it to work for a few minutes, then empty the whole into a dish of water, and carefully wash. Remove the sections with a glass rod into another vessel of water, and thence into water on an object slide; there they can be torn to pieces with needles.

(2) Put the sections in a tube with an equal bulk of chlorate of potash, cover with concentrated nitric acid, and proceed as above.

(3) Use 1 gram. chlorate of potash to 50 cc. nitric acid, and proceed as above.

(4) 3 grains chlorate of potash and 2 drachms nitric acid (sp. gr. 1·10); keep the sections in this, cold, for a fortnight.

After carefully washing in alcohol, the preparations as above can be preserved in glycerine.

Magenta. See Fuchsin.

Magnesia sulphate. Used in culture fluid for Algæ, 208.

Marjoram, Oil of. Clearing reagent, before mounting in dammar or Canada balsam, 365.

Methyl-blue (Methylene-blue). Watery solution. Preparations washed, after staining, in water, show the cell-wall coloured, and not the protoplasm. Sections of alcohol-material must be washed in water before staining.

Methyl-green. Alcoholic solution, used for material in absolute alcohol. Stain for 5–30 minutes, wash in distilled water, and mount in glycerine. Stains nuclei only.

Methyl-green, Acetic. *See Aniline-green, Acetic.*

Methyl-green, Formic. Dissolve methyl-green in 1–2 per cent. of formic acid till a deep blue colour is produced.

Methyl-violet. A concentrated alcoholic solution should be kept. For use, it can be added drop by drop to a little distilled water till this is deeply coloured. This fluid is specially used for staining the pellicle (zooglœa) of bacteria. A portion of the pellicle is placed on an object-slide, and a drop or two of the methyl-violet placed thereon and allowed to remain till the stain appears sufficiently deep. A little experience is needed here to judge the strength of the fluid and depth of the staining. If this is too deep, the jelly in which the bacteria are embedded is also stained. After staining, wash the preparation with water, or, better, with a 10 per cent. solution of acetate of potash. After lying for half an hour in the air, the preparation can be mounted in Canada balsam; not in glycerine, as that dissolves out the colour. Or it can be mounted in a watery (10 per cent.) solution of acetate of potash.

Methyl-violet in sulphuric acid. Dissolve methyl-violet in strong sulphuric acid till brownish-green; then add water slowly till violet. This swells cell-walls and stains protoplasm. Hence it is very useful for sieve-tubes. Stain the preparation, and then wash with water.

Methyl violet, Watery solution. Used as stain for chromatophores, 39, 41.

Millon's reagent. Dissolve metallic mercury in its weight of concentrated nitric acid, and dilute with an equal volume of distilled water. This reagent ought to be prepared fresh. Swells cell-walls, and displays their lamination. Protein substances are disorganized, but, after standing for a while, take a characteristic brick-red colour. Slightly warming hastens this. (This reaction appears to fail sometimes.)

Molybdate of ammonia. Dissolved in a concentrated solution of ammonium chloride.

Mounting fluid. *See Enclosing fluid.*

N.

Nigrosine (Quality I. of Trommsdorf). Watery solution.

Nigrosine, Picric. To a saturated watery solution of picric acid, a small quantity of watery solution of nigrosine is added till the fluid appears a deep olive-green colour. Exceedingly good for unicellular and filamentous algæ, staining and fixing at the same time (12–24 hours).

Nitric acid. Used in xantho-proteid reaction, *q. v.*, and for maceration.

O.

Osmic acid, 1 per cent. *Must* be kept in darkness, and in a well-closed bottle. Colours oil-drops brown. Instantaneously fixes living protoplasm, and hence serves in nuclear studies. In a mixture of 9 parts 0·25 per cent. chromic acid solution, and 1 part 1 per cent. osmic acid, filamentous algæ, *Nitella*, etc., can be at the same time hardened and stained.

P.

Phenol. *See* Carbolic acid.

Phloroglucin. Alcoholic (or watery) solution, 1 to 5 per cent., or even so low as $\frac{1}{10}$ per cent., will do. Followed by hydrochloric acid, is the best reagent for lignin; *see* p. 59. Phloroglucin is expensive, but a convenient substitute can be prepared from cherry-wood. *See* Cherry-wood, extract.

Picric acid. Saturated watery solution.

Picric alcohol. Picric acid dissolved to saturation in 50 per cent. alcohol.

Picric aniline-blue. To a saturated watery solution of picric acid, about 4 per cent. of a saturated watery solution of aniline-blue is added, so that a deep blue-green fluid is produced.

Picric nigrosine. *See Nigrosine, Picric.*

Potash (Potassium hydrate). Concentrated watery solution. A test for suberin (p. 155). Suberized tissues placed in it become yellow; if warmed, the colour is much deeper; if boiled, the suberin is exuded in the form of yellowish drops.

Potash, Dilute watery solution, 5 or 6 per cent. Specially used as a "clearing" reagent.

Potash, Acetate of. Concentrated solution in water. Used as a mounting fluid. After covering with the cover-glass, the preparation must not be sealed for about 24 hours. The fluid does not crystallize. *See* pp. 172, 177.

Potash, Alcoholic (Russow's). Rectified spirit is mixed with a concentrated watery solution of potassium hydrate till a slight precipitate is formed. The fluid is frequently shaken, and allowed to stand for 24 hours. The resulting weakly yellow fluid is poured off from the sediment, and for use must be diluted with half its volume of distilled water. For the ordinary purposes of dilute potash this is preferable to the watery solution.

All these potash reagents, especially the watery solutions, must be kept in well-stoppered bottles, and the stopper occasionally anointed with vaseline.

Potash, Bichromate of. 10 per cent. solution in water. Reagent for tannin.

Potash, Chlorate of. Constituent of Schultze's macerating mixture.

Potash, Nitrate of. A constituent of culture-fluid for algæ, 208. 10 per cent. solution, causing plasmolysis, 37*a*.

Potassio-sodic tartrate. Used in Fehling's sugar reaction, 48.

R.

Rosaniline violet (Hanstein's).—Equal parts methyl-violet and fuchsin (majenta) mixed and dissolved in alcohol. Shows stratification of cell-walls, and differentiates sections of stems, especially monocotyledons. Stains protoplasm bluish-violet; amyloid substances, nucleus, and gums different shades of red; resins, blue; tannin, foxy-red; cellulose, pale violet; lignin, reddish; bast-fibres, deep red; sieve-tubes and bast parenchyma, hardly at all.

S.

Safranin in alcohol (absolute). Saturated solution. Stains nucleus well in material hardened in alcohol, chromic acid, or picric acid. In the latter two cases the sections must first be washed in water, and then placed in a

little of the saffranin solution diluted with an equal quantity of distilled water. Leave in this from 12 to 24 hours. They can be examined from time to time in a drop of the same fluid to see how the staining is proceeding. Then wash in absolute alcohol till they cease to give off colour; place in oil of cloves or of marjoram, and then mount in dammar or Canada balsam.

Safranin in water. To differentiate stem of *Lycopodium*, 149.

Schultze's fluid. See Chlorzinc iodine.

Schultze's macerating mixture. See Macerating mixture.

Shellac. The clearest possible, dissolved in absolute alcohol to the thickness of syrup, and filtered. A mounting medium, 365.

Soda, Caustic. Solution of sp. gr. 1·12. Used in Fehling's sugar reaction, 48.

Sodium chloride (table salt). Used in culture-fluid for algæ, 208.

Sodium sulphite, 232. Used, warmed, as a solvent for sulphur-grains.

Sugar (Cane). For solutions.

Sulphuric acid, concentrated.

Sulphuric acid, dilute. Equal volumes of sulphuric acid and water. Also 2 acid : 1 water.

T.

Turpentine.

V.

Vaseline.

Vesuvin. Water solution. Used for staining bacteria, 230.

W.

Wax. Small wax candles.

X.

Xanthoproteid reaction, for protoplasm. With successive use of nitric acid and ammonia, protoplasm colours yellow.

Xylol.

APPENDIX IV.

General Notes on Methods and Selected Reagents.

The following notes include the most useful of the reagents given in Appendix III., and those therefore which it is desirable should be included in a limited selection. They are classified according to their purpose or nature.

For alcohol and methylated spirit, see a note at the beginning of Appendix III.

Micro-chemical Reagents.—These are used on account of certain effects (reactions) which they produce upon some constituent or other of the plant, and by which therefore the presence and nature of that constituent can be recognised. Naturally in micro-chemistry we are restricted to optical reactions, and hence these reagents always produce distinctive effects, either swelling, dissolving, colouring, evolution of gas, etc.

(1) *Iodine in alcohol.*
(2) *Iodine in glycerine.* Can be made from (3) when required.
(2*) *Iodine in chloral hydrate.*
(3) *Potassium-iodide iodine.*
(4) *Chlorzinc iodine.*
(4*) *Chlorzinc iodine and iodine.*

Acids.

(5) *Sulphuric acid (concentrated).*
(6) *Sulphuric acid (dilute,* 2 acid : 1). Can be prepared from (5).
(7) *Hydrochloric acid (concentrated).*
(7*) *Hydrochloric acid* (½ per cent. in 70 per cent. alcohol).
(7†) *Hydrochloric acid* (¼ per cent. in 70 per cent. alcohol).
7* can be prepared when wanted by putting 1 drop strong acid in a watch-glass of 70 per cent. alcohol. 7† requires great care in use. *See* p. 365.
(8) *Nitric acid* (strong).
(9) *Acetic acid* (glacial).
(9*) *Acetic acid* (1 per cent.).
(10) *Chromic acid* (concentrated).
(10*) *Chromic acid* (25 per cent.). Other percentages can be made from this as required.

Alkalies.

(11) *Ammonia* (strong).
(12) *Potash* (concentrated).
(12*) *Potash* (5 or 6 per cent.).
(12†) *Potash in alcohol.*

Inorganic Salts.

(13) *Acetate of copper* (crystals). Used in Barfoed's sugar reaction.
(13*) *Sulphate of copper* (crystals). Used in Fehling's sugar reaction.
(14) *Perchloride of iron.*
(15) *Ferrous sulphate* (crystals).
(16) *Bichromate of potash* (10 per cent. watery solution).
(17) *Chlorate of potash* (crystals).
(18) *Potassio-sodic tartrate.*
(18*) *Caustic soda.*

Organic bodies.

(19) *Alcohol* (absolute, or 90 per cent.).
(20) *Methylated alcohol.*
(21) *Ether.*
(22) *Alcanna* (Alkanet) root, or tincture.
(23) *Aniline chloride* (in alcohol).
(24) *Camphor.*
(25) *Carbolic acid* (phenol).
(26) *Diphenylamin.*
(27) *Gelatine.*
(28) *Phloroglucin*, or *Cherry-wood extract.*
(29) *Cane sugar.*
(30) *Turpentine.*

PRESERVING FLUIDS.—The best is *methylated alcohol*, of which a good supply should be kept. The material to be preserved for future work should be completely covered. For work in nucleus or protoplasm. *absolute alcohol* must be used.

SOFTENING.—Alcohol material, especially if old, is often brittle, and stems, etc., are hard. Soften for 24 hours in—

(31) *Half-and-half glycerine and meth. alcohol.**

HARDENING.—Soft tissues can be hardened for cutting by 3 days in methylated spirit.

FIXING THE CELL-CONTENTS.—For studies in nuclei or protoplasm it is necessary to fix the cell-contents of the material, while quite fresh, without allowing them to contract. The material must be in very small pieces, so as to be rapidly permeable, and the fluid, except in the case of osmic acid, must be 100 times the bulk of the material. The best fixing fluids are:—

(19) *Absolute* (or at least 90 per cent.) *alcohol.*
(32) *Chromic acid*, 0·5 per cent.
(32*) *Chromic acid*, 1·0 per cent., or *chrom-acetic acid*, 1·0 per cent.
(33) *Osmic acid*, 1·0 per cent.
(34) *Picric acid* (saturated watery).

In chromic acid, chrom-acetic acid, or picric acid of the above strengths the material can lie for 24 hours; then be laid in distilled water for any time up to 24 hours. If not required for use at once, instead of this, first wash in 50 per cent. alcohol, then transfer to 70 per cent., and finally to absolute alcohol, or strong methylated spirit, changing this after 24 hours if it is then discoloured. In this they can be kept for use.

Most algæ are best fixed in picric acid, etc., mixed with an equal volume

* See Note *a* on page 404.

of alcohol, for the same reason that alcohol potash is often preferable to watery potash solution, viz., to avoid undue swelling of the walls.

SECTION-CUTTING.—Various hints on this subject will be found scattered in the text; especially *see* pp. 16 and 52. For other references *see* "Sections" in the General Index. Where the material to be cut is alcohol-material, keep the razor and surface wet with alcohol; otherwise use water or glycerine. The razors are best hollow-ground; but for cutting wood use a razor only slightly hollowed. Keep the razors sharp.

Thin objects which have to be cut can be placed in glycerine-gum, between pieces of elder-pith or smooth cork; or even, if moderately hard, between pieces of soft wood, such as poplar or lime-wood.

Very small objects may be embedded, either in celloidin or glycerine-gum (see these headings in Index). For very minute objects, such as pollen-grains, gum alone, or with a very small proportion of glycerine, can be used. A layer of strong gum is placed on the end of a piece of elder-pith, and when set nearly firm the pollen-grains dropped on it, and then another drop of gum placed on the top.

For cutting with a microtome, *see* p. 63.

Sections when cut may be lifted from the razor with a camel-hair brush. If laid on flatly, they will not curl. With such a brush a section can likewise be turned over. *See* p. 17.

CLEARING.—The cell-contents are often opaque; and when it is desired especially to see the cell-walls, as, *e.g.* in growing-points, the preparation must be cleared. Clearing reagents act by dissolving, or at least swelling, the cell-contents. The best of these are—

(25) *Carbolic acid.*
(12) *Potash* (5 or 6 p.c. watery).
(12†) *Potash, Alcoholic.*
(11) *Ammonia.*
(35) *Chloral hydrate.*
(36) *Eau de Javelle.*

After treatment with chloral hydrate, watery potash, or ammonia, wash in water; after alcohol potash, in alcohol, and then mount in glycerine. Eau de Javelle is specially used for alcohol material. For method, *see* p. 172. After treatment, place in dilute alcoholic glycerine, and allow this slowly to concentrate.

Sections of alcohol-material which have been stained and are to be mounted in Canada balsam or dammar, must be cleared in another way. First place them for a minute or two in alcohol of the strength of that used for staining, and then into absolute alcohol for another like period. Thence transfer to—

(37) *Oil of cloves,*
(38) *Oil of marjoram,* or
(39) *Turpentine and creosote* (4 : 1).

Sections stained with aniline dyes should be treated as above, but cleared in—

(40) *Oil of cedar.*

STAINING.—This may be done in a watch-glass, or, better still, in small white

earthenware saucers, such as are sold with cheap paint-boxes, could these be obtained of larger size, say 1¾ or 2 in. in diameter. As a rule, the best staining is obtained by dilute solutions and long treatment. The section to be stained must be immersed in the staining fluid, and examined from time to time to judge progress.

The object of staining is to show up (differentiate) diversities of structure, either of the plant skeleton or of the cell-contents, each stain particularizing some special feature.

For a limited list, selected from those in Appendix III., the best are :—

(41) *Hoffmann's blue.*
(42) *Acetic aniline-green.*
(43) *Corallin.*
(44) *Fuchsin* (Magenta) in 50 per cent. alcohol.
(45) *Acetic gentiana-violet.*
(46) *Methyl-violet in alcohol.*
(46*) *Methyl-violet in sulphuric acid.*
(47) *Safranin in alcohol.*

(These are all aniline dyes, stain rapidly, and the preparations fade if kept in the light.)

(48) *Thiersch's borax-carmine.*
(48*) *Beale's carmine.*
(49) *Böhmer's* or *Grenacher's logwood* (best old).

In many cases two stains can be used concurrently ; such are :—

(50) *Fuchsin iodine-green.*
(51) *Picric aniline-blue.*
(52) *Picric nigrosine.*
(53) *Rosaniline violet.*

MOUNTING.—Preparations can be mounted in

Glycerine.
Glycerine-jelly.
Hoyer's mounting fluid (for aniline preparations).
Mounting fluid (for logwood and carmine preparations).
Acetate of potash.
Canada balsam in turpentine.
Dammar.

No preparations containing water or glycerine can be mounted in Canada balsam or dammar. Watery preparations must be first dehydrated in alcohol ; glycerine preparations first soaked for some time in water, and then in alcohol.

For mounting in glycerine-jelly or Hoyer's fluid, water or alcohol must previously be removed by laying for some time in glycerine. *See also* p. 206.

Preparations in acetate of potash retain their chlorophyll.

CLOSING.—Preparations mounted in glycerine-jelly, Hoyer's fluid, Canada balsam, or dammar do not need further closing. In all these cases it is however, desirable to put one or two thin layers of—

Gold-size with a fine brush, over the junction of cover-glass and object-

glass. This must not be done till the mounting fluid has set firm; and one layer of gold-size should be dry before another is added.

Preparations in glycerine should be hermetically sealed with Canada balsam in turpentine, laid round the cover-glass thinly with a thin glass, rod, and, when dry, topped with gold-size. *See* p. 92.

Preparations in acetate of potash should be closed with gold-size.

LABELLING.—Paper labels may be stuck on the object-slides with *pure gum*; Card labels, on the other hand, with *glass cement*, such as Crystal Palace cement, coaguline, etc.

The following is a still more restricted list, answering nearly all practical purposes:—

MICRO-CHEMICAL REAGENTS.—Iodine in chloral hydrate, Potassium-iodide iodine, Chlorzinc iodine, Sulphuric acid (conc.), Hydrochloric acid (conc.), Nitric acid (strong), Acetic acid (1 p.c.), Chromic acid (25 p.c.), Ammonia (strong), Potash in alcohol, Potash (conc.), Sulphate of copper (crystals), Perchloride of iron, Bichromate of potash (10 p.c.), Chlorate of potash (crystals), Potassio-sodic tartrate, Caustic soda, Ether, Alcanna tincture, Carbolic acid.

PRESERVING FLUID.—Methylated alcohol.

FIXING AND HARDENING FLUIDS.—Absolute alcohol, Picric acid (in 50 p.c., meth. spirit).

CLEARING FLUIDS.—(Carbolic acid, Potash in alcohol, Ammonia,) Eau de Javelle, Oil of Cloves.

STAINS.—Hoffmann's blue, Corallin, Acetic gentiana-violet, Thiersch's borax-carmine, Logwood, Picric aniline-blue.

MOUNTING MEDIA.—Glycerine, Glycerine-jelly, Acetate of potash, Canada balsam, Gold-size, Gum.

STAINS.—Hoffmann's blue, Saffranin, Acetic gentiana-violet, Thiersch's borax-carmine, Logwood, Picric aniline blue, magenta.

MOUNTING MEDIA.—Glycerine, Glycerine-jelly, Acetate of potash, Canada balsam, Gold-size, Gum.

[*Note to page* 401.]

a Softening dried material can be effected by means of a solution of 1 part caustic potash in 5 parts water, to which 5½ parts glycerine are then added.

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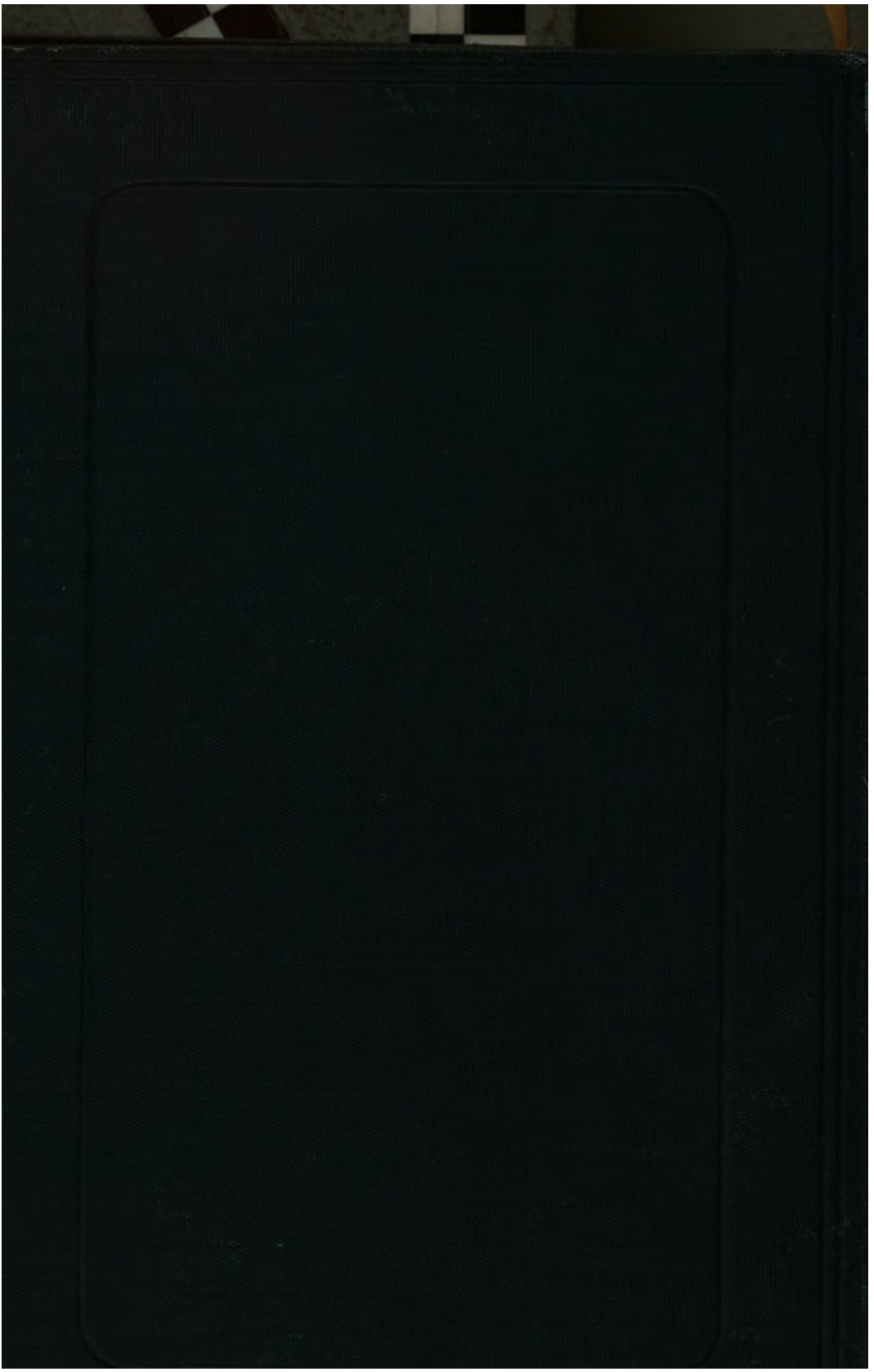
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